

PAPERS.

11. On the Structure and Function of the Gas Glands and Retia Mirabilia associated with the Gas Bladder of some Teleostean Fishes, with Notes on the Teleost Pancreas. By W. N. F. WOODLAND, F.Z.S., The Zoological Department, University College *, London.

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(Plates II.-IX.† and Text-figures 53-62.)

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INTRODUCTORY.

In the present paper it is proposed to give a fairly complete account of the gross and histological structure of the vasculo-glandular organs or "red bodies" found in the wall of the gas bladder ‡ of many teleost fishes, and to offer certain suggestions

* The greater part of the work connected with the present paper was carried out in the Zoological Department of King's College, London.

† For explanation of the Plates see pp. 245-248.

‡ The terms "swim-bladder" and "air-bladder" are inappropriate, since fishes do not use this apparatus for swimming and it does not contain air.

respecting the physiology of these organs. Though the literature* concerned with the morphology and physiology of the gas bladder is, of course, very considerable, yet up to the present only one author (64, 65) has attempted a complete review of the subject of the present paper in the light of recent work. Further, although there exist several memoirs dealing with special types of "red body," yet most of these are so occupied with cytological and other details that, in the opinion of the present writer, they have failed to supply such an account and such illustrations of the gross structure of these "red bodies" as to render evident some of their most remarkable features. Finally, the Jaeger-Nusbaum controversy calls for an independent description of these unique organs, so interesting from the physiological standpoint.

I have divided the present paper into two parts, the first of which deals with the structure and the second with the physiology of the "red bodies." With reference to the drawings illustrating Part I., I have, with a few exceptions, adopted a definite scheme of coloration in order to facilitate comprehension. Green has been adopted to denote the internal lining epithelium of the bladder, and therefore the glandular, *i. e.* gas gland, epithelium is always of this colour in the drawings, since this is but a special development of the usually squamous lining epithelium; red denotes blood; black or grey has been employed for connective and muscular tissue and secretion material; and yellow has been used to indicate the pancreas, which is often closely associated in a mechanical sense with some parts of the "red body." The sources and methods of preparation of my material are stated in Appendix A (p. 236).

PART I.—THE STRUCTURE OF THE "RED BODIES" IN VARIOUS TELEOSTEI.

It is necessary to recognize at the outset that the term "red body" unfortunately has been applied to different structures by no means equivalent to each other: thus, *e. g.*, the "red bodies" of the Common Eel are simply the two retia mirabilia, whereas the "red body" of the Cod consists both of numerous small retia mirabilia and of the large mass of epithelial cells called the gas gland, the retia and the gas gland being, in this case, intimately associated. A gas gland exists in the Eel as in all other fish with "red bodies," but it is not in close connection with the rete mirabile as in the Cod. This being the case, it must be understood that by the term "red body" all that is meant is a red mass situated in the bladder-wall, the constitution of which is variable. I may add that in the vast majority of cases "red body" includes both rete mirabile and gas gland. As an introduction to the study of gas gland structure I shall first describe the anatomy of one of the most simple as well as most common

* For the literature and historical reviews of the entire subject of gas bladders up to 1866 see Milne-Edwards (49) and Gouriet (34); for more recent literature see the lists supplied by Bridge (22), Hüfner (38), Jaeger (44), Deineka (29), Reis & Nusbaum (62, 63), and at the end of the present paper.

forms, viz. that of the Common Eel, *Anguilla vulgaris*. The "red body" of the Eel has been described several times previously, *e. g.* by Jacobs (42, 43), Quekett (61), and Corning (28), but never, I venture to think, in a sufficiently clear or comprehensive manner and certainly never adequately illustrated.

The Vasculo-glandular Structure in the Bladder of the Common Eel
(*Anguilla vulgaris*).

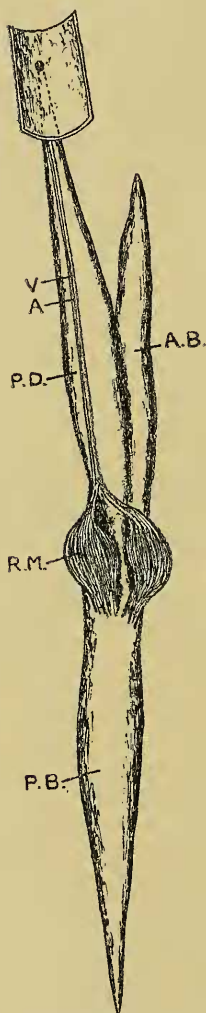
It is well known that the bladder of the Eel is an elongated sac lying dorsally and towards the posterior end of the body cavity and connected with and opening into the œsophagus by means of a long wide duct—the ductus pneumaticus (text-fig. 53, P.D., p. 186). The pneumatic duct opens into the bladder at about midway in its length, the bladder in consequence being divisible into anterior (A.B.) and posterior (P.B.) regions. The bladder and duct are both situated in the same horizontal plane, *i. e.* the duct opens into the bladder at the side, not dorsally or ventrally. Running along the ventral side of the duct are an artery and a vein situated close together (A.V.). This artery and vein give rise to the two ovoid retia mirabilia (R.M.) where the duct opens into the bladder, the two retia lying one on each side of the duct and therefore being dorsal and ventral in position respectively and in the same vertical line.

The wall of the bladder (see Pl. II. figs. 1-6, *e. g.*) in fish generally is best described as consisting of the tunica externa and tunica interna. The former consists of two definite layers of fibrous tissue, the outer being loose in texture, often containing muscular tissue and varying greatly in thickness, the inner dense, silvery in appearance, and principally consisting of elastic fibres. The latter consists of a thin basis of dense connective tissue, with which is closely connected the innermost layer of the bladder wall, a layer consisting usually of flattened cells. It is this innermost cellular layer which gives rise to the mass of glandular cells in connection with a rete mirabile—the gas gland. The tunica interna is easily separable from the tunica externa, and it is advisable to remove this latter before cutting sections of the gland.

The vascular supply of the bladder may first be considered. It has already been mentioned that an artery and a vein run along the ventral side of the wall of the ductus pneumaticus. These two vessels are situated in the outer fibrous layer of the tunica externa (Pl. II. fig. 1), which is here of considerable thickness, and they supply several arteries to the duct and receive several veins from it. Shortly before these two vessels reach the region of the junction of the duct with the bladder, the artery and the vein each divide into two (fig. 2), so that there are two pairs of vessels instead of one, each pair consisting of an artery and a vein. Now the artery and vein of each of these pairs undergoes further subdivision, the smaller arteries and veins resulting from this subdivision intermingling in their course, but not once communicating with each other, so that we now have situated towards

the upper and lower sides of the ductus pneumaticus two distinct masses of intermingled arteries and veins (fig. 3), each mass having resulted from the subdivision of the two pairs of arteries and veins, which, as we have seen, have themselves originated

Text-fig. 53.



The bladder, duct, and "red bodies" of *Anguilla vulgaris*,
viewed from the side (after Quekett).

P.D., pneumatic duct which opens anteriorly into the œsophagus; A and V, artery and vein supplying bladder; R.M., rete mirabile; A.B., anterior region of bladder; P.B., posterior region of bladder.

by the division of the initial artery and vein. The subdivision of the arteries and veins in each mass proceeds rapidly, until there is formed by this process an ovoid mass of extremely fine parallel arterial and venous capillaries, closely intermingled with each other, but never intercommunicating and indistinguishable structurally as arteries and veins under the highest powers of the microscope (fig. 4). These two ovoid masses of parallel arterial and venous capillaries lying on the dorsal and ventral sides of the ductus pneumaticus just anterior to its junction with the bladder are the retia mirabilia of the eel-bladder (text-fig. 53, R.M.).

The finest arterial and venous capillaries of each rete mirabile run parallel to each other for a short distance, but soon they commence to unite, arterial capillaries with arterial capillaries and venous with venous, in order to re-form larger vessels similar to those which produced the capillaries by subdivision. This process of reunion proceeds so far as to form two masses of large intermingled arteries and veins similar to those depicted

Text-fig. 54.



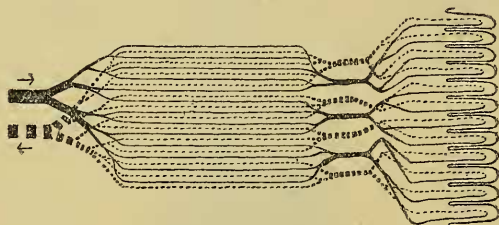
External aspect of a rete mirabile (after Quekett). The anastomoses of the vessels shown in this figure do not occur in actuality.

in figure 3 (*cf.* figs. 3 & 5). When reunion has proceeded thus far, each of these masses of re-formed arteries and veins once more breaks up into fine capillaries in connection with the vascular supply of the glandular folded epithelium which lines the bladder internally. I reproduce here as faithfully as I am able (text-fig. 54) Quekett's figure (61) of the construction of the Eel's rete mirabile, which illustrates in a general way that which I have just described. Text-fig. 54 is unsatisfactory in that it does not indicate the fact that the vessels and capillaries forming the rete are of two kinds—arterial and venous—closely intermingled with each other, although never intercommunicating; also anastomoses never occur between adjacent large vessels in the manner shown in the figure. A more satisfactory diagram of the construction of the Eel's rete mirabile is the one I have

designed for text-fig. 55. In this the artery and the vein giving rise to a rete mirabile are distinguished from each other by the latter being cross-striated, the subdivision of each of them is indicated, also the intermingling of the fine parallel capillaries, their reunion and the second breaking up into capillaries of the large arteries and veins thus formed to supply the epithelium of the gas gland.

Up to the present I have spoken of the arterial and venous capillaries or vessels composing each of the two retia mirabilia merely as tubes, but if, of course, we take into account the direction of the flow of blood in the two sets of vessels, then it is evidently incorrect to speak of the arteries and veins both subdividing at the anterior pole of the rete mirabile to produce

Text-fig. 55.



R.M.

G.E.

The construction of the rete mirabile bipolare geminum (R.M.).

G.E., glandular epithelium of gas gland.

capillaries: what is really happening in this region is that the arteries are subdividing and the veins uniting (text-fig. 55). That this is the actual mode of construction of the rete mirabile is not only proved by the study of serial sections but also by the injection method. And here let me remark upon the extraordinary process implied by this construction of the rete mirabile—a term which, like its German rendering, “wunder-netze,” happens to be peculiarly appropriate*. It is implied that parallel vessels carrying blood in opposite directions can so influence each other, or be so influenced by a common cause, as to break up into a rete mirabile at the same spot, the subdivision of each of the two sets of vessels being so adapted to the other as to ensure the close apposition and intimate intermingling of their finest capillaries†. It is evident that this intricate

* Müller (53) termed this type of rete mirabile a “rete mirabile bipolare geminum,” in contradistinction to a “rete mirabile bipolare simplex,” such as the purely arterial carotid gland of Amphibia.

† The fact illustrated by these retia that blood-vessels are able to capillarize on their own account, *i.e.* quite apart from any other tissue, first attracted my attention to these “red bodies” when engaged in certain speculations concerning the “renal-portal system” in Vertebrates (76, 77). The formation of the retia seems to lend support to Dr. Shore’s views (68, 69) concerning the vascularization of the liver as distinguished from the venous penetration of the kidney-substance to form the “renal-portal system.” I hope shortly to investigate the subjects of the “renal-portal system” and the various kinds of retia mirabilia from a practical standpoint.

mechanism must be for some definite purpose, especially since it is found in all fishes possessing gas glands. What that purpose may be is a question I shall discuss later (see Part II.).

The epithelium lining the ductus pneumaticus, which is supplied with a few branches direct from the bladder artery and is therefore not supplied by the vessels connected with the retia mirabilia, is quite simple in character. It merely consists of a single layer of flattened cells raised up at intervals by the large capillaries running in the basis of connective tissue underlying the epithelium (Pl. II. fig. 7). These capillaries in the wall of the duct apparently have much the same surface arrangement as those found in the lungs of certain reptiles (Quekett, '61), but it is hard to suppose from this fact alone that the epithelium of the duct is normally respiratory in function, since the blood supplied to this has already been oxygenated in the gills; on the other hand, it is equally improbable that the arterial blood in the wall of the duct serves to alter the composition of the adjacent gas since there is reason to suppose that the duct normally serves as a mere exit for the superfluous gas of the bladder (Jaeger, '45). It is possible, however, that this large vascular supply of the duct epithelium in the Eel is really correlated with the terrestrial habits of the animal, since when an Eel travels across a meadow the gills are useless for obtaining oxygen, and under these circumstances the animal doubtless draws upon the oxygen in the bladder—the duct acts as a lung. Moreau proved that Perch, when placed in water previously boiled so as to deprive it of dissolved air, utilized the bladder-oxygen for purposes of respiration. An interesting confirmation of this view is that in other physostomes (*Myrus*, *Ophichthys*, *Esox*) the duct epithelium is, as we shall see, practically devoid of capillaries*.

—The lining epithelium of the bladder proper is very different in character from that of the duct. It consists of a more or less folded single layer of cells which are large in size and short columnar in form, slightly granular, and, when stained by the picro-indigo-carmin method (Appendix A), usually rather opaque (Pl. II. fig. 8). The folding of this layer is due to the presence of large capillaries which push their way inwards towards the bladder-cavity from the connective tissue base, and in so doing cause the epithelial layer to form corresponding emergences. Thus the interior of each projection of the glandular epithelium into the bladder cavity is occupied by an extension of connective tissue enclosing capillaries. The spaces situated between such emergences adjacent to each other are also regarded as forming gland ducts. The folding of the epithelium is very variable in different parts of the bladder wall, in some parts almost disappearing and in others being more accentuated than that depicted in figure 8; this folding is much more marked in the anterior region of the bladder than in the posterior. It might appear to be significant that immediately the lining epithelium of the duct becomes supplied with blood connected with the rete mirabile system of vessels

* I find that Jacobs (42, 43) states this view as a fact.

(that is, when the duct opens into the bladder) it then assumes the glandular folded character of the bladder epithelium, were it not for the fact that in other physostomous genera this sharp distinction between duct- and bladder-epithelium is absent, as I shall show shortly.

The intracellular structures described in detail below as being present in the cells of the gas glands of other teleost fishes—intracellular gas bubbles, capillaries, and ducts—are not easily to be detected in my preparations of the Eel, but I believe I have seen gas bubbles present in a few cases, also the capillaries occasionally intrude somewhat upon the cells at their bases. Granular matter, ejected into the bladder-cavity with the gas, is so small in amount in the gland ducts of my preparations that it may be said to be absent.

The folded glandular epithelium of the gas gland of the Eel, and presumably of all other genera possessing a folded epithelium, originates from a single unfolded layer which during development becomes pushed out into the folds by the activity of the large capillaries situated in the thin layer of connective tissue lying at the base of the epithelium*.

Thus in the type of "red body" found in the Common Eel we have two large retia mirabilia, situated some distance from the epithelium of the bladder which they supply, and the greater part of this lining epithelium of the bladder is glandular and folded in a simple manner to form the gas gland. In all "red bodies" we have these two elements, the rete mirabile and the gas gland, but in the majority of "red bodies" these two elements are much more intimately associated with each other and more complex in form as compared with the structure of the simple type of "red body" just described. The Eel type of "red body" serves as a convenient starting-point for considering more complex types.

Before considering these types, it is necessary to point out a serious mistake contained in almost the only English paper dealing with the present subject, that of Vincent and Barnes (75), which, though the paper is now quite out of date, has found its way into at least one recent text-book ('Cambridge Natural History,' vol. vii. 1904, p. 308). Vincent and Barnes, following Coggi (25), state that "red glands are only found in those bladders which are devoid of a ductus pneumaticus," and they draw a sharp distinction between Physoclisti, which possess the gas gland, and Physostomi, which are supposed to be devoid of one. I need hardly say that no such distinction exists. Vincent and Barnes are right, in so far as they say that the "red bodies" of the Eel are only partially comparable with those of the Gadidae (*vide supra*), but it is difficult to understand how they came to overlook the large and conspicuous glandular epithelium lining the bladder cavity. Further, as Deineka (29) points out, in the physostomous Pike (*Esox lucius*) a conspicuous gas gland

* I am much indebted to Dr. W. G. Ridewood for kindly presenting me with most of my young material.

exists (described by Coggi, 26), comparable in all essential respects with that of the Cod. Also Vincent and Barnes, unlike many of their predecessors, altogether fail to appreciate the peculiarity of the arterial and venous nature of the rete mirabile; indeed, they even appear to have some doubt as to the fact itself. They say: "Corning has discriminated between arteries and veins in what we have usually spoken of as the 'capillary masses.' This he has done by means of a series of injections. . . . This method would certainly give some idea of the distinction between the smallest arterioles and smallest venules, but we are doubtful whether it would be more than a very rough method of distinction, that it would be, in any given case, an infallible test as to whether a particular small vessel were to be called artery or vein. Much would depend, it appears to us, on the strength of the injecting force [1]. However this may be, we have failed to make out anything in the minute structure which would warrant us in dividing up the 'capillary masses' into arterioles and venules." This statement suggests that Vincent and Barnes never worked through a series of sections of the Eel (or any other similar) "red body" (which possibly also accounts for their missing the glandular epithelium of the Eel, which, as above stated, lies separate from and posterior to the retia mirabilia*), since the arterial and venous nature of the rete mirabilia is rendered quite evident by this means alone; also the last-quoted statement of Vincent and Barnes is still less comprehensible when we remember that these "red body" retia mirabilia have been correctly described in full and compared with the several other kinds of retia mirabilia which exist by Johannes Müller (53) in 1840, not to mention Owen's description (not confirmed by his figure, however) of the "red body" of the Eel just referred to †.

✓ *Vasculo-glandular Structures of the Anguilla or First Type in the Bladders of some other Anguilliformes.*

MYRUS VULGARIS Kaup.

In this physostome the vascular and glandular conditions are similar to those found in *Anguilla vulgaris*. The only essential distinction between the two is in the character of the lining epithelia of the duct and bladder, and in this particular the

* A fact clearly stated by Owen ('The Comparative Anatomy and Physiology of Vertebrates,' vol. i. 1866, p. 495): "The two chief 'retia mirabilia' or vaso-ganglions, in the air-bladder of the Eel and Conger, which are situated at the sides of the opening of the air-duct, are also 'bipolar' and consist of both arterioles and venules [curiously enough, however, in contradiction of this statement, the figure 329 of the 'vaso-ganglion' of the Eel supplied on p. 496 shows the blood going through all capillaries in one direction!]; they consist of straight parallel capillaries, as in fig. 329; their afferent trunks do not ramify in the immediate margin of the vaso-ganglion from which they issue, as in the vaso-ganglions of the Cod, Burbot, Acerine, and Perch, but run for some distance before they again branch to form the common capillary system of the living membrane of the air-bladder."

† These criticisms must not be held to imply any lack of appreciation of a very lucid and interesting paper; the fact, however, that the statements criticised have been made use of by the late Professor Bridge so recently as 1904 shows the need for correction.

distinction is striking. In *Anguilla* the epithelia of the duct and bladder are, as we have seen, quite different in character; in *Myrus vulgaris*, on the other hand, they are very similar. The duct epithelium consists of a single layer of short columnar cells which is only slightly folded. Very few capillaries are present in the connective tissue layer underlying the epithelium, though here and there large capillaries are to be seen in transverse section. Thus the duct is not lined by squamous epithelium as in *Anguilla*. In the bladder of *Myrus vulgaris* the epithelium is, as just stated, similar to that of the duct, the only difference being that the cells are larger. The folding of the epithelium is at most very slight and most pronounced in the region of the opening of the duct; in the anterior and posterior regions of the bladder, folding of the epithelium is practically absent. There is never any approach to the degree of folding seen in *Anguilla*. It is also noticeable that where the epithelium passes over a large capillary (and capillaries are not very numerous) the cells immediately assume a more squamous character.

OPHICHTHYS (SPLEGEBRANCHUS) IMBERBIS Delar.

In this physostome the plan of construction of the "red body" is essentially the same as that found in *Anguilla* and *Myrus*, but there exists in the single specimen at my disposal one modification in connection with the rete mirabile. When the bladder is viewed *in situ*, a single "red body" is seen to be present which is situated anteriorly in the ventral wall where the duct joins the bladder. A series of transverse sections shows that this "red body" is, as in the Eel, a large rete mirabile, which, however, is disposed in a different manner in the present instance. The artery and vein run along the right side of the bladder and break up to form the rete in such a manner that the long axis of this lies transversely to the length of the fish (Pl. II. fig. 9). The capillaries of the rete mirabile unite as usual to form larger vessels, which here, of course, lie to the left side of the bladder, where they break up anew to supply the epithelium of the gas gland. As in *Myrus*, there exists no sharp distinction between the glandular epithelium of the duct and that of the bladder, also the capillaries in connection with the epithelia are comparatively few in number. This epithelium consists, as in the two preceding genera, of a single layer of more or less columnar cells which occasionally becomes very slightly folded, but over the greater part of the wall, both of the bladder and the duct, the epithelium is a simple unfolded layer of columnar glandular cells. Strange to say, the cells of the epithelium of the bladder in my preparations are distinctly smaller than those of the duct. Occasionally large capillaries are present in the bladder wall, and where these occur the epithelial cells become, as in *Myrus*, almost squamous in shape. Small capillaries also exist, though they are not numerous. The epithelium of the duct remote from the bladder becomes much more squamous in character than elsewhere.

OPHICHTHYS (OPHISURUS) SERPENS L.

The general plan of the *two* retia mirabilia and the gas gland is exactly that found in *Anguilla*. The epithelial lining is, as in *Anguilla*, squamous and covers projections of the subjacent connective tissue, which contain, not one large capillary, but many small ones. Posteriorly the lumen of the duct, which anteriorly is circular in transverse section, becomes very restricted and star-shaped in outline, owing to the great size of the connective tissue projections just mentioned—a feature not met with in the species described above. The epithelium of the bladder wall is hardly folded at all over the greater part of its area, simply consisting of a single layer of short columnar glandular cells, but in places folding occurs, occasionally to such an extent as to resemble the bladder epithelium of *Anguilla*, but this is rare.

It is curious how the epithelial linings of the duct and bladder differ in these four closely related fish—*Anguilla*, *Myrus*, *Ophichthys imberbis*, and *O. serpens*. In the duct and bladder of *Anguilla* we have the extremes of flattened and folded epithelia; in the other genera intermediate conditions of the epithelium exist, both as regards the form of the cells and their arrangement, and the duct and bladder epithelia are not sharply distinguishable from each other, and yet the vascular supply has the same distribution in all cases, although it undoubtedly varies considerably in amount, being much greater in the Eel than in the other genera. The different conditions of the rete mirabile in the two species of *Ophichthys* are also noticeable.

The type of vasculo-glandular apparatus found in the bladders of the preceding genera may be defined as follows: the glandular epithelium is composed of a single layer of cells which either remains unfolded or is only simply folded, and the retia mirabilia are one or two in number and are large and distinctly separate from the gas gland, the capillaries of the rete reuniting to form large arteries and veins before coming into connection with the glandular epithelium.

*A Second Type of Bladder Vasculo-glandular Apparatus,
as exemplified in Syngnathus acus Mich.*

Syngnathus acus is a physoclistous fish in possession of a "red body," which is, on the whole, very similar in construction to that of the Eel, but which nevertheless exhibits certain differences which justify us in separating it as a distinct type. The "red body" in *Syngnathus* is visible with the naked eye as a small red patch at the extreme anterior end of the bladder. An artery and vein running in the anterior attachment of the bladder divide up in the manner already described for the Eel to form the single rete mirabile (Pls. II. & III. figs. 10–14). It will also be observed in figures 10 and 11 that posterior extensions of the diffuse pancreas (indicated in the figures by yellow masses) penetrate into the anterior end of the rete mirabile and, as will be shown in detail

later, are closely associated with the larger veins. Lying alongside the rete mirabile is the anterior extremity of the elongated closed bladder (fig. 14), the wall of the anterior region of which is very glandular and vascular. A little way behind the anterior extremity of the bladder, the side of the rete next the bladder comes into connection with the bladder epithelium (fig. 15), the capillaries uniting (*i. e.* the arterial capillaries are uniting and the venous capillaries are really here subdividing to form the minute venous capillaries of the rete) to form slightly larger vessels before supplying the epithelium of the gas gland. This process of supplying the glandular epithelium goes on until the whole of the rete mirabile is used up (Pl. III. figs. 15-17), the fine capillaries of the rete always uniting to some extent to form larger vessels before these supply the epithelium. The bladder epithelium, as shown in the figures, becomes enormously thickened by a process of folding during the breaking-up of the rete mirabile. Posterior to the rete mirabile, which has thus disappeared in supplying the glandular epithelium, this latter itself gradually dwindles (fig. 18) until in the posterior region of the bladder the cavity is lined with simple squamous non-glandular cells.

The epithelium of the bladder is quite simple in type and essentially resembles the folded epithelium of the Eel, though the folding is much more pronounced. Figure 19 represents the glandular epithelium in the unthickened region, that is, lining any side of the bladder cavity other than that adjacent to the rete mirabile, where, as just stated, the epithelium is much thickened. The folds of the epithelium due to the large capillaries which push out the epithelium are so deep that we have here, as in *Anguilla*, distinct ducts formed between the folds which contain on occasion large quantities of floccular matter evidently derived from the cells. The thickened epithelium adjacent to the rete mirabile is formed by the great extension of the folds just mentioned, the cells of which often join at intervals, *i. e.* the folds run together so that the at-first-separate ducts form anastomosing channels before opening into the bladder. The cells forming this epithelium are faintly granular and almost cubical in form, and here and there contain gas bubbles (not shown in the figure) and intracellular capillaries, but since I have examined these structures in greater detail in other types, I shall not refer to them any further at present. I shall also, and for the same reason, merely mention here the conspicuous opacity of the cytoplasm of the gland cells which comes into immediate contact with a capillary (fig. 19).

One other fact which is very well shown in my slides of *Syngnathus* remains to be mentioned, and that is the disintegration of the red blood corpuscles contained in the capillaries of the rete mirabile and the gas gland first into spherical globules and ultimately into granular matter. This granular matter resulting from erythrocytolysis is, of course, only found in the blood-stream and is to be carefully distinguished from the granular matter above described as being found in the bladder lumen and gland

ducts just external to the cells of the gas gland. Figure 21 (Pl. III.) shows several stages of this disintegration of the erythrocytes, the significance of which I shall discuss later.

Figure 20 represents a highly-magnified transverse section through the fine arterial and venous capillaries composing the mass of the rete mirabile. The two kinds of capillaries are quite indistinguishable by mere inspection. The capillaries are separated from each other by a small amount of connective tissue. In the very young fish, *i. e.* before the capillaries have subdivided to the extent found in the adult, the arterial capillaries are distinguishable from the venous, the former being thicker-walled and somewhat smaller in calibre (see also Bykowski & Nusbaum, 23).

Thus the chief features of the second or *Syngnathus* type of "red body" are that the glandular epithelium is arranged in simple folds, that it is restricted in area, not lining the whole of the bladder cavity, and that the rete mirabile is contiguous with the gas gland, although a small amount of reunion of the capillaries of the rete may occur before these supply the epithelium.

Other Examples of the Syngnathus Type of "Red Body."

GوبيUS NIGER.

As another example of the second or *Syngnathus* type of bladder vasculo-glandular apparatus I shall now describe that of *Gobius niger* caught at Naples*. The "red body" of *Gobius* is a small oval red patch situated just anterior to the centre of the oval outline of the bladder (Pl. III. fig. 22). It consists of a single rete mirabile which supplies a small area of glandular epithelium. As seen in a series of transverse sections, the artery and vein, which break up to form the rete, at first lie *outside the several layers of the bladder wall* (Pl. IV. fig. 23) together with large masses of pancreas. The artery and vein subdivide as usual to form a cluster of small arteries and veins, which in *Gobius*, as in *Syngnathus*, are intermingled with masses of pancreas (fig. 24). It will also be noticed that this cluster of arteries and veins is at this stage in process of *breaking through the thin dense outermost layer of the bladder wall*, so as to lie within this latter, large masses of pancreas being included with the cluster of vessels. Still more posteriorly when the arteries and veins have undergone subdivision to a greater extent, the whole mass of vessels together with the included portions of pancreas have become almost entirely *included within the bladder wall*, small portions of pancreas only being left outside (fig. 25). Finally, when the rete mirabile is completely formed it is wholly included within the substance of the bladder wall, as in previous genera (fig. 26). The rete mirabile supplies the adjacent area of glandular epithelium

* *Gobius paganellus*, obtained from Plymouth, possesses a star-shaped "red body" resembling that of *Peristethus* in construction (Pl. V. fig. 37, and text-fig. 57), though less definite in outline. *Gobius minutus*, also obtained from Plymouth, has a similar star-shaped "red body," but the gas gland epithelium in some specimens is hardly folded at all, merely consisting of a single layer of cells enclosing large capillaries; in others it is folded as in *Gobius niger* (Pl. IV. fig. 27).

in the usual way, the epithelium lining the rest of the bladder remaining squamous in character. It is, however, noticeable that in the rete of *Gobius* the fine capillaries do not unite together at all to form larger vessels before supplying the glandular epithelium (see fig. 27). In *Syngnathus* it has been mentioned that the reunion of the fine capillaries is very slight, but here it is quite absent.

The glandular epithelium is of the same type as that found in *Syngnathus*, the cells only, as may be seen from figure 27, being somewhat more columnar in form. The epithelial cells of *Gobius* are relatively large, and, as just stated, columnar in form and in my preparations faintly granular. They are folded in the simple manner already described for *Syngnathus* and *Anguilla*, large capillaries being situated in the interior of the folds*. In figure 27 are represented three epithelial folds of the gas gland, underlying which are a few of the multitudinous fine capillaries composing the rete mirabile. Even in this figure it can be observed that several of the cells contain spherical clear spaces and that two small capillaries have encroached on the space previously occupied by the cytoplasm of two cells of the gas gland. These intracellular bubbles and capillaries and the intracellular lumina, which I shall also shortly mention, are cytological features which have, within the last year or so, been studied in great detail by Jaeger (44-47), Bykowski & Nusbaum (24), and Reis & Nusbaum (54, 55, 62-66), and they are of considerable interest from several standpoints. I shall discuss their significance later; for the present I may remark that I think I am justified in redescribing these structures, especially in view of the controversy concerning the mode of gas-production considered in Part II. That the fine capillaries of the rete mirabile often find their way deep into the substance of the glandular cells, as well as between them, is proved in *Gobius* by a very moderate amount of inspection under a high power of the microscope (Pl. IV. figs. 28, 29). It is also noticeable that the protoplasm of that border of the glandular cells in contact with the capillary develops a dark and often striped appearance, which Nusbaum & Reis suggest is due to diffusion streams of nutritive matter passing from the blood into the gland-cells, but which I believe is intimately concerned with the production of the gas contained in the bladder (see Part II.). Concerning the presence of vacuolar spaces in the cytoplasm of the cells of the gas gland, nobody can doubt their existence who has examined well-preserved preparations of gas glands which are *in an active condition*. These vacuolar spaces are with good reason assumed to represent bubbles of gas being generated within the cell-substance (see Part II. and Appendix B), but it is evident that only on occasion will the gas gland be fixed and preserved when active; in the majority of cases, preparations of gas glands will not exhibit these intracellular gas bubbles, simply because the gas

* Reis (64) states that the folds branch and anastomose in *Gobius*; in all my preparations of *Gobius niger*, *Gobius paganellus* and *Gobius minutus* (also in *Gobius capito*—Coggi, 25) the folds are quite simple, as described in the text.

gland is normally quiescent*. To deny the existence of these cytoplasmic bubbles simply because certain microscopic preparations do not show them is illogical, to say the least, since intracellular gas bubbles, unlike intracellular capillaries, are not permanent but transitory structures. Of over fifty series of preparations which I have made of numerous types of gas gland, not more than seven or eight show these intracellular gas bubbles in an unmistakable manner. In the type of gas gland under consideration, one only of my six or seven series of preparations of the gas glands of different specimens of *Gobius niger* and *G. paganellus* exhibits a few gas bubbles (fig. 27). I have seen these bubbles best in a preparation of *Gobius minutus* (Pl. V. fig. 35), in which the gas gland cells were fixed in the active condition. As shown in figure 35, the majority of the gas-producing cells possess large vacuolar spaces in their cytoplasm usually present in the vicinity of the nucleus; indeed, the nucleus is often so adpressed as to assume a crescentic form. These vacuoles, which, as just mentioned, are assumed to represent the moulds, so to speak, which contained gas bubbles, just as liver- and kidney-cells exhibit similar vacuolar spaces which contained liquid globules (text-fig. 60, p. 225), are of various sizes, and some can be seen in the act of being ejected from the cell substance into the bladder lumen, where they are also occasionally found in a liberated condition (see Appendix B). The bursting of these gas bubbles is doubtless accountable for the masses of granular matter always found, when the gland is active, in the bladder lumen and gland ducts just external to the glandular epithelium, this granular matter, of course, having composed the walls of the bubbles. The gland cells, when active, always have that portion of their cytoplasm situated next vascular tissue very distinct from the rest, it being, as already described, very dense and often striated in appearance; the rest of the cytoplasm usually assumes a "stringy" appearance and is very vacuolate†. It may also be mentioned that the nucleus is not situated in the dense cytoplasm next the blood, in which respect gas gland cells show a marked difference from the cells of the pancreas, *e. g.*

In addition to intracellular capillaries and gas bubbles there are also occasionally to be seen intracellular lumina or ducts—continuations of the intercellular ducts into the substance of the gland cells. These, again, vary greatly in different types of gas gland and in different specimens of each type. In none of my preparations are they very numerous, and doubtless, like the gas bubbles, the smaller intracellular lumina are transitory structures. I have figured one or two of these structures in connection with the gas gland of *Ophidium barbatum*, a type very effectually studied by Reis & Nusbaum (62), and they are occasionally to be found in my preparations of *Gobius niger* and *Gobius paganellus* opening into the numerous finer intercellular ducts. Since these

* See Addenda (1).

† For excellent descriptions and figures of these gas bubbles in the cytoplasm of the gland cells, see the papers of Bykowski & Nusbaum (24) and Reis & Nusbaum (55, 62, 63).

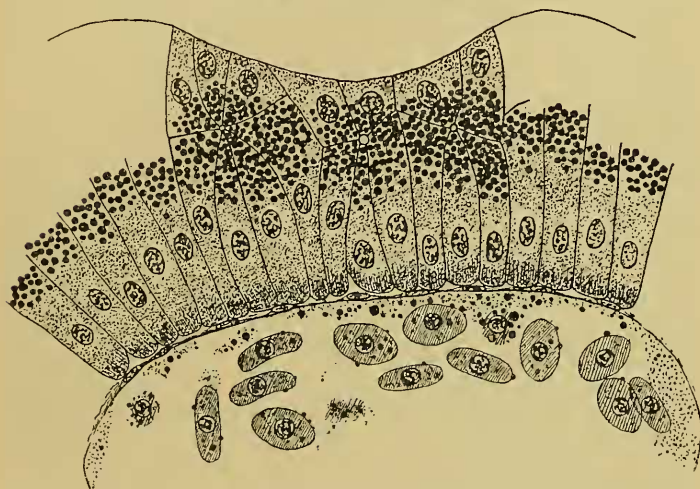
intracellular channels have been so thoroughly studied by the authors just named, I shall not refer to them in any detail. I will merely say that since, according to Jaeger (47), gas glands are able, when active, to swell to three times their normal size, and that the finer ducts only become visible when filled with fluid, it is not surprising that they are only visible in very few preparations.

One or two other histological features to be observed in serial sections of the gas gland and associated structures of *Gobius niger* and other types remain to be mentioned. In transverse sections across the anterior end of the rete mirabile, and only at its anterior end, where the arteries are easily distinguishable from the veins, all the arteries possess a peculiar endothelium. This endothelium (Pl. IV. fig. 32) contains many more nuclei than usual, and these, instead of being flattened in the usual way, are more or less spherical, and, each being contained in a small mass of cytoplasm, project into the vascular lumen in the manner shown in the figure. This peculiar endothelium, in the case of the smaller arterial capillaries, results in such a thickening of the wall (and incidentally in some of the smallest capillaries a blocking-up of the lumen) as to cause the capillary to bear a strong resemblance to a bile- or pancreatic duct; indeed, were it not for the presence of blood corpuscles these small capillaries would be almost unrecognisable as such. This type of vascular epithelium, which must be well-known to histologists, in all probability results from the contraction of the pulsatile arteries in forcing the blood through the rete, the narrowed circumference of the endothelium causing the cells both to assume a globular form and to protrude into the lumen of the vessel.

Concerning the posterior extension of the diffuse pancreatic acini among the arteries and veins anterior to the formation of the rete mirabile (fig. 25, *e. g.*) there is little to note other than the fact itself, which I have not seen recorded by writers on the teleost pancreas (see list of references to pancreas literature below). When first studying teleost "red bodies" I was misled into supposing that this extraordinary extension of the pancreatic acini through and right into the bladder wall in many teleost genera represented a new gland specially developed in connection with the rete mirabile. I suggested (78) that the purpose of this supposed new gland was the abstraction from the venous blood stream of the globules and granules resulting from the breaking-up of the red blood corpuscles referred to above, and in my sections (stained by the picro-indigo-carminic method described in Appendix A) the zymogen granules present in the pancreas cells often strongly resemble erythrocyte globules which have been abstracted from the blood stream (*cf.* text-fig. 56 and Pl. III. fig. 21). Adopting the view of Jaeger (fully discussed in Part II.) that the cells of the gas gland produce a toxin for the purpose of breaking up the erythrocytes and so enable themselves to absorb more easily the oxygen in the blood stream which they subsequently pump into the gas bladder, I suggested that the purpose

of the rete mirabile—a structure essentially consisting of the intimate juxtaposition of the veins returning the blood from the gas gland and carrying the hypothetical toxin, with the arteries carrying freshly-oxygenated blood to the gas gland—was to allow the toxin in its venous capillaries to diffuse into its arterial capillaries and so to disintegrate the oxygen-laden erythrocytes in time for the oxygen to be available for abstraction by the gas gland cells by the time the arterial blood reached the gas gland. On this view the veins of the rete mirabile would be laden with granular matter (as indeed they are—see fig. 32) which requires to be eliminated, and I suggested that this process of elimination was the function of these pancreatic masses which are so closely connected with the veins at the anterior end of the rete—the kind

Text-fig. 56 ($\times$ circ. 470).



Vein surrounded by modified acini (three shown) of the pancreas in *Nerophis equorius*.

of vessels and the identical position that the hypothesis would suggest; in other words, the hypothesis was supported by the position of the gland, the modification of the pancreatic acini surrounding the veins (described below), the great similarity between the erythrocyte globules and granules in the blood, the zymogen granules in the pancreatic cells and the granules in the pancreatic ducts, and the *à priori* necessity for the elimination of the erythrocyte granular matter. This hypothesis, however, concerning the function of what I subsequently recognized as the pancreas is obviously untenable, since the zymogen granules are of course present in pancreatic cells not associated with the rete mirabile veins and, as in higher Vertebrates, do not occur outside the pancreas cells; also the posterior extension of the teleost

pancreas into the bladder wall cannot be supposed to be of any more significance than its penetration into the liver. I failed at first to recognize this gland as the pancreas, both because of its peculiar position inside the bladder wall and because of the peculiar modification of the pancreatic acini in juxtaposition with the veins, neither of which peculiarities have been described by writers on the teleost pancreas. Another small peculiarity existing in several teleost genera (*Gobius niger*, e. g.) which I studied more than others was the syncytial character of the wall of the pancreatic duct (Pl. V. fig. 33 and Pl. IV. fig. 34), a feature not present in most teleosts. The modification of the pancreatic acini in connection with the veins (veins from the rete mirabile as well as hepatic-portal veins from the gut wall) just referred to consists of the cells of each acinus next the thin vein wall being drawn out, the cells on the side remote from the vein being quite short, as shown in text-fig. 56. This figure also indicates the similarity between the zymogen granules and the erythrocyte globules, both globules and zymogen granules being stained a bright emerald-green in my preparations. As stated in Part II., I now have good reason to believe that the erythrocyte granular matter is abstracted from the blood in part by the liver and in part by special cell-masses situated near the kidneys. Laguesse (4-10), Rennie (17), and others have shown that the teleost pancreas resembles in all essentials the pancreas of higher Vertebrates—in the characters of the acini, ducts, zymogen granules, and the presence of centro-acinal cells and areas of Langerhans. With respect to these last, I may mention incidentally that I can fully confirm the results of Rennie (17) and there can be but little doubt now that these areas constitute an organ physiologically and in many teleosts anatomically distinct from the pancreatic acini. This view of the physiological independence of the islets of Langerhans is supported by the researches of Diamare (3), Flint (3a), Opie (14, 15), de Witt (2), Lane (11) and others in opposition to the view that the islets are patches of exhausted pancreas, supported by Lewaschew (12), Laguesse (6, 10), and Dale (1); and, as I have already remarked, researches on the teleost pancreas indicate quite plainly that the former is the correct view.

SIPHONOSTOMA TYPHLE (RONDELETHI Delar.), NEROPHIS
ÆQUORIUS.

The "red bodies" of these two genera are, like that of *Syngnathus*, small red patches situated at the extreme anterior end of the bladder and identical in their plan of construction. The only particular in which the "red body" of *Siphonostoma* differs from that of *Syngnathus* is that in the former the cells of the gas gland are much larger and the intervening ducts much narrower; indeed, in the posterior portion of the gland the lumina are so narrow as not to be seen very easily. The large capillaries are also more circular in transverse section in *Siphonostoma* than in *Syngnathus*. Gas bubbles and intracellular capillaries are in my preparations to be found occasionally in the substance of the

epithelial cells, also the cytoplasm in contact with the capillaries is darkened in the usual way. A small amount of granular material is present in the bladder lumen and gland ducts just external to the cells. I must also mention a peculiar class of corpuscles found in the blood of my specimen of *Siphonostoma typhle*. These peculiar corpuscles (Pl. V. fig. 36) possess very dense cytoplasm, are about half the size of the red corpuscles, and contain large nuclei. They are fairly numerous, forming roughly about 1 per cent. of the total number of corpuscles. They doubtless represent white corpuscles, though in my preparations they to some extent resemble small rounded ganglion cells; indeed, they bear quite a strong resemblance to the large dense ganglion cells so often found between the pancreatic acini, and are in consequence very conspicuous. I have not observed such conspicuous white corpuscles in the blood of any other of my preparations.

In *Nerophis* the gas gland epithelium is folded like that of *Gobius niger* and possesses the same features.

GASTEROSTEUS SPINACHIA.

In this Stickleback the only feature in which the "red body" differs from those of the last four genera described is the splitting-up of the rete mirabile into three or four bunches supplying the gas gland epithelium at different levels; instead of the initial artery and vein wholly dividing up to form a single rete mirabile, an artery and a vein are given off from this to form a rete mirabile in connection with the most anterior region of the gland epithelium, another pair to form a second rete mirabile in connection with the middle region of the gland epithelium, and, finally, the initial artery and vein themselves form a third rete mirabile to supply the rest of the gas gland. This condition in *Gasterosteus* is intermediate between that described for *Syngnathus* and that about to be described for *Peristethus*. In all other respects the "red body" of *Gasterosteus* resembles the *Syngnathus* type.

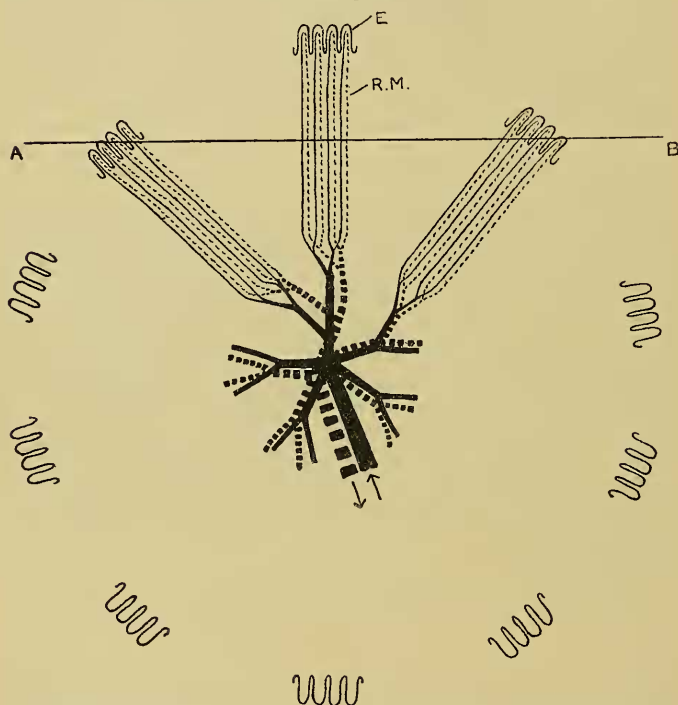
PERISTETHUS CATAPHRACTUS (PERISTEDION CATAPHRACTUM C. V.).

The "red body" of *Peristethus* is star-shaped (Pl. V. fig. 37), owing to the fact that the artery and vein which supply the glandular epithelium, when arriving at the centre of the star-shaped "red body," break up each into ten vessels. The score of vessels thus formed sort themselves out into pairs of vessels, each pair consisting of an artery and a vein. These pairs radiate out from the point of subdivision of the original artery and vein like the spokes of a wheel and give rise to ten elongated retia mirabilia in the manner shown in text-fig. 57. Each rete mirabile supplies a radially-disposed tract of glandular epithelium developed as usual from the innermost cellular layer of the bladder wall. The capillaries of each rete mirabile coalesce to a certain extent to form larger vessels before supplying the gas gland (not indicated in

text-fig. 57), but not to the same extent as in *Syngnathus*. Figure 38 (Pl. V.) represents a section (A-B in text-fig. 57) across the anterior end of the star-shaped "red body."

The epithelium of the gas gland is folded as in *Gobius niger*, but, as in *Syngnathus*, it becomes considerably thickened by the elongation and fusion of the folds in the vicinity of the rete mirabile. The cells are rather small and short-columnar in form, and, in the particular specimen which I examined, contained numerous gas bubbles (figs. 39, 40, 41); in other words, the gland was here in a highly active condition when preserved.

Text-fig. 57.



The construction of the "red body" of *Peristethus cataphractus*.

E., epithelium of gas gland; R.M., rete mirabile. Fig. 38 (Pl. V.) represents a section taken across A-B.

The most remarkable feature about the "red body" of *Peristethus* is, of course, the radial disposition of the divisions of the rete mirabile and the glandular epithelium; in every other respect it appertains to the *Syngnathus* type as above defined.

TRIGLA HIRUNDO (CORAX Bp.).

The bladder of *Trigla hirundo*, when observed from the ventral

aspect, appears as an elongated sac, the ventral wall of which is very muscular in the median line posteriorly. At the sides of this muscular posterior median portion of the bladder wall there are to be seen two red streaks, which are the lateral divisions of the "red body" (Pl. VI. fig. 42). In a series of transverse sections it can be seen that the cavity of the bladder has a peculiar conformation*: anteriorly the bladder cavity consists of two laterally-placed channels, which end blindly anteriorly and proceed posteriorly over a distance equal to about one-third the length of the bladder when each divides into two; these four channels thus formed then extend to about midway in the length of the bladder, when the two inner channels coalesce to form a wide median chamber, which, with the two external channels, extends to the posterior extremity of the bladder (Pl. V. fig. 43). It will be observed in figure 43 that two great median bands of muscle (M.B.) are developed in the ventral wall of the posterior half of the bladder, and this explains why it is that only the lateral "red bodies" are seen when the bladder is viewed from the ventral aspect (fig. 43). Also shown in this figure are the two laterally-placed strands (S) of nerve-fibres developed in connection with the two muscle-bands.

The epithelium of the gas gland is folded in the manner characteristic of this type of "red body," but in *Trigla* it is more folded than in previous examples (Pl. VI. fig. 44), the primary folds bearing small lateral folds. In some places the depth of the folding is increased as in *Syngnathus*. The cells are of moderate size and columnar in form. The rete mirabile is divided up into capillary tufts, *i. e.* there are numerous small retia mirabilia which supply the epithelium at intervals in its course; in other words, pairs of vessels are given off at intervals from the bladder artery and vein, each pair consisting of an artery and a vein and forming a rete mirabile to supply a certain area of gas gland.

TRIGLA GURNARDUS.

Concerning the gas gland epithelium of this species, see p. 207, below.

SMARIS MAURII, SMARIS VULGARIS.

The "red body" of *Smaris vulgaris* has the macroscopic appearance shown in figure 45 (Pl. VI.). Figure 46 (Pl. V.) represents diagrammatically a transverse section across the anterior end of the "red body," the primary artery and vein entering at the centre of the oval mass.

Concerning the character of the gas gland epithelium of these two species (Pl. VI. fig. 47), see p. 207, below.

Previously described "Red Bodies" of the Syngnathus Type.

The glandular epithelia of the "red bodies" of *Gobius capito*, *Trigla gurnardus* (see my description below), and *Gasterosteus*

* See description in Milne-Edwards (49).

aculeatus have been described and figured by Coggi (25); *Gasterosteus aculeatus* has also been described and figured by Deineka (29); *Blennius*, *Gobius*, *Syngnathus*, and other types have been briefly described by Reis (64). All these "red bodies" evidently belong to the *Syngnathus* type as defined above.

*A Third Type of Bladder Vasculo-glandular Apparatus,
as exemplified in Ophidium barbatum L.*

The "red body" of *Ophidium barbatum* has received a considerable amount of attention during the last few years, especially from the cytological standpoint (Bykowski & Nusbaum, 24 and Reis & Nusbaum, 62, 63), since the huge cells composing the gas gland offer exceptional facilities for enquiry in this direction. But, as in some other instances, good figures of the gross anatomy do not exist, so far as I am aware, and hence I shall make no apology for recapitulating already-known facts in a manner that shall be intelligible to the reader who has not worked at the subject.

The "red body" of *Ophidium barbatum* is situated anteriorly in the ventral wall of the bladder and has the curious appearance shown in figure 48 (Pl. VI.). The fan-shaped deep red portion is, of course, the rete mirabile, which is here an undivided body, and it is formed by an artery and vein which enter at the pointed posterior apex. The pink margin surrounding the anterior border of the rete is, obviously, the gas gland. A transverse section running mid-way through the "red body" exhibits the parts diagrammatically indicated in figure 49 (the outer layers of the bladder have been stripped off). In the centre is the rete mirabile and at the sides are the enormously thickened glandular portions of the (elsewhere) flattened lining epithelium. As in some former instances, there is no reunion of the capillaries of the rete mirabile before supplying the glandular epithelium: they directly supply the gas gland.

Now in this type of "red body" the gas gland does not consist of a single layer of cells which has become folded, but of a many-layered mass of cells, the arrangement of which bears no resemblance to the folded condition of the single-layered epithelium of the "red-bodies" already described (see figs. 54, 58, 63 for examples of the massive type of gland), and, judging from the development of this massive type of gas gland epithelium as seen in *Atherina*, it never does, at any stage of its development, bear a resemblance to the folded type of gland. The many-layered or massive kind of gas gland epithelium, as seen in *Ophidium*, *Atherina*, and other genera to be mentioned, is, then, a distinct type; the question raised by Reis in a recent paper (64) as to whether there exist forms of bladder epithelium transitional between these two types is one which I shall discuss when describing *Atherina*. The faintly-granular cells composing this many-layered gas gland of *Ophidium* are of quite moderate dimensions in the vicinity of the rete mirabile, but become larger the further they are removed, and the cells at the remote edge of the gland are remarkable for their large size. In this type of gland there exist

no large ducts comparable with those we have seen in the glands consisting of folded epithelium; on the contrary, the only ducts visible are narrow slit-like channels occasionally to be detected in their course between the cells. These narrow channels vary in width greatly according to the state of activity of the gland; they are necessarily numerous (though by no means always to be observed) in order to provide means of exit to the surface for all the numerous cells concerned, and they open on the gland surface inside the bladder lumen by small pores. Also penetrating the mass of the gland are numerous fine capillaries supplying the individual cells with nutriment and oxygen on the sides remote from those facing the ducts just mentioned. All the intracellular structures previously mentioned as occurring in connection with cells of the gas gland are here shown in a very obvious manner. Figure 50 (Pl. VI.) shows intracellular lumina, one in longitudinal section. As to the significance of these intracellular passages found in gas gland cells, it is not necessary to regard them all as possessing purely a nutritive utility as Nusbaum & Reis have supposed; it seems more probable, judging from their appearance in such cases as that illustrated in figure 50, that some at least are concerned with the passage of the gas bubble from the interior of the cell to the exterior; they are very rare in their occurrence in my preparations and in transverse section closely resemble bubbles, especially if near the nucleus. The whole mass of cells composing the gas gland is therefore penetrated by capillaries and gland lumina. I have not observed a pancreas in the region of the rete in *Ophidium*.

Other Examples of the Third or Ophidium Type of "Red Body."

BOX BOOPS L.

The "red body" of *Box loops* has the curious macroscopic appearance shown in figure 51 (Pl. VI.). Figure 52 (Pl. VII.) is a diagram of a transverse section through the middle of the "red body," and the appearance of two concentric loops is thus seen to be due to the disposition of the rete mirabile. The primary artery and vein enter the "red body" posteriorly and break up into smaller arteries and veins (intermingled with pancreatic acini) to form the rete mirabile as usual. The glandular epithelium consists, as in *Ophidium*, of a mass of cells interpenetrated by capillaries and lumina, but here the cells are all approximately equal in size and the gas gland is more than a dozen cells thick.

ATHERINA HEPSETUS L.

In macroscopic appearance the "red body" of *Atherina hepsetus* is diffuse in form (Pl. VI. fig. 53) and consists of tufts of retia mirabilia borne laterally on a median pair of vessels (artery and vein) with corresponding tracts of glandular epithelium. The glandular epithelium (Pl. VII. fig. 54) is very similar to that of *Box boops*; in other words, it consists in its thickest parts of a mass of small cells, seven or eight cells in thickness, with inter- and

occasional intra-cellular ducts and capillaries. I have not observed a pancreas in the region of the rete. The cell-outlines of the gas gland are faint and a small amount of granular material is present in the bladder lumen in my preparation.

I stated above that the massive type of gas gland is quite distinct from the folded type in certain genera, and, indeed, these two types of bladder epithelium are almost always easily distinguishable from each other in the genera which I have studied: genera, with a few exceptions to be mentioned, either possess the folded or the massive type of gland and not glands intermediate in structure. In young specimens of *Atherina* about 15 mm. in length, that portion of the bladder epithelium which is about to produce the gas gland is at first composed of a single layer of cubical or short columnar cells, and it is worthy of notice that these cells, in order to build up the fully-formed massive gland, all divide at right angles to the plane of the bladder wall (Pl. VII. fig. 55), *i. e.* the successive planes of cell-cleavage are parallel with the plane of the bladder wall—the initial unilaminar epithelium never becoming folded at any stage of development. As the gas gland cells multiply connective tissue cells may be observed to extend in between them in order to furnish the connective and vascular tissues which penetrate the mass of the gland when fully formed. Also if figures 54 and 55 be compared, it will be seen that the cells (especially the nuclei) of the gas gland of the adult *Atherina* are noticeably smaller as compared with those in the young fish. The fact that there is no indication of folding of the bladder epithelium during development of the massive type of gland is important, since Reis (64) supposes, on the ground that there exist forms of bladder epithelium more or less intermediate in structure between the folded and massive types, that the latter has been derived from the former, and if such be the case we might expect that the massive type of gland in its development would pass through a folded developmental stage. As we have seen, this is not the case in *Atherina* and presumably not in other genera possessing typically massive glands. I assume this with the more confidence since in some gas glands which, owing to the exceedingly close apposition of the folds, at first sight appear to be massive, it is always possible to refer them at once to the folded type by observing in each such case the margins of the gland where it merges into the squamous epithelium lining the greater part of the bladder cavity, since here the folds are quite simple: such marginal folds are never present in true massive glands.

Reis, in contending that the massive type of gland is but a derivative of the folded type, instances as transitional forms between the two types the gas gland epithelia of *Syngnathus* and *Girardinus*. I have not examined *Girardinus*, but the gas gland epithelium of my specimens of *Syngnathus* can certainly not be said to be transitional, since, as my description above of this epithelium clearly shows, the massiveness of the gland merely results from the extreme length of the folds and their occasional anastomosis:

the glandular epithelium is always unilaminar and the folds always clearly recognizable. My preparations do not at all confirm Reis's statement that the tubular outgrowths are so numerous at the base of the gland that they lose their lumina through mutual contact, and form almost complete layers of epithelial cells. On the other hand, the gas glands of some other genera which I have examined certainly do appear to be transitional in structure between the folded and massive types, viz. those of *Trigla gurnardus*, *Smaris maurii*, and *Smaris vulgaris*. I may say at once that in all three species, especially *Trigla gurnardus* and *Smaris maurii*, the gas gland shows distinct signs of being of the folded type, and in the two species just mentioned the folds are quite distinct and unmodified at the edges of the glands, but, on the other hand, the surface of the gland is covered by a continuous single layer of columnar epithelium (never present in the typical folded gland) and throughout the mass of the gland the cells at certain points lose their unilaminar arrangement*. In *Trigla gurnardus* †, and perhaps also in the other two species, the explanation of the superficial cell-layer seems to be that at the edges of the gland the unilaminar epithelium splits into two layers, the upper remaining unfolded and forming the superficial cell-layer and the lower becoming folded in the usual manner (text-fig. 58), the folds, however, anastomosing to a considerable extent and, as

Text-fig. 58.



Diagram to explain the probable construction of the gas glands of
Trigla gurnardus and *Smaris maurii*.

already mentioned, the cells in places becoming clustered into groups more than one layer in thickness. In *Smaris maurii* (Pl. VI. fig. 47) the individual cells are larger than in *Trigla gurnardus* and the anastomosing folds more closely packed; here and there the cells are several layers thick, but in general they are arranged in single folded rows as in *Trigla gurnardus*. In *Smaris vulgaris* the epithelium has almost entirely lost its folded character, though this is sometimes to be detected at the edges of the gland. Thus the gas glands of these three species ‡ are to a certain extent

* In many massive glands the cells are often in places arranged in single rows, and this is evidently due to the necessity (which Oppel points out: see footnote on page 208) of each cell being in contact on one side with a duct and on the other with a blood vessel.

† Coggi (25) provides a figure of the epithelium of this species showing a simple folded character; the epithelium in my specimens more resembles his figure of the epithelium of *Motella mediterranea* with the addition of a superficial covering layer of columnar cells.

‡ And possibly those of *Polyacanthus* (*Macropodus*) described by Reis & Nusbaum (62) and *Motella* described by Coggi (25).

intermediate in character between the folded and massive types, but we may still retain these two categories, since we have reason to suppose that all of these three supposed transitional forms of gas gland really belong to the folded type and that they have only secondarily acquired features characteristic of the massive type by the splitting-off of a superficial cell-layer covering the surface of the gland, by close apposition and anastomosis of the elongated folds, and by occasional local proliferation of the cells; in other words, until their development is known, we may provisionally distinguish between truly or primarily massive glands which never exhibit the folded arrangement of cells at any stage of development, and pseudo- or secondarily-massive glands which are secondarily derived from the folded type*. To me Reis's suggestion that all massive glands have been derived from folded glands seems very improbable, since on this view the folded condition should occur as a stage in their development, and we have seen that this is not the case in at least one typical genus (*Atherina*). It seems more probable that both folded and massive types have originated independently from the simple unilaminar condition which must in all cases form the starting-point of development both in ontogeny and phylogeny.

CORIS JULIS † (VULGARIS Flem.).

The "red body" of *Coris julis* has the circular shape seen in figure 56 (Pl. VI.), and the rete mirabile supplying the gland is noteworthy for the way in which it is broken up into small divisions (Pl. VII. fig. 57). The glandular epithelium (fig. 58), which is

* Reis's classification of gas gland epithelia (64) seems to me confirmatory of this view. Her classification is as follows: (1) entirely unilaminar epithelium folded into simple tubular outpushings; (2) the epithelial folds are much folded and branched [Reis has made the mistake of including in this group the gas gland epithelium of *Coreina*; in my preparations of *Coreina nigra* the epithelium is of the most typical massive type]; (3) the epithelium is in parts folded and in parts multilaminar [I cannot agree to the inclusion of *Syngnathus* in this category. I have not examined Reis's other example—*Hippocampus*]; (4) the compact or massive glands of *Sargus*, *Charax*, and other genera. Reis regards these four types as a developmental series—a mistake I have corrected in the text. Reis also remarks that only in the first three types are the ducts obviously the spaces between the epithelial folds or tubular outgrowths; in the fourth there exist no such intertubular ducts, intercellular crack-like channels only being present. This last statement seems to me to be confirmatory of my contention.

Reis makes another statement which, as Oppel (58) points out, contravenes all probability. This statement is that whereas in the folded type of gland the individual gland cell only presents one surface towards the duct lumen (the others being contiguous with other cells and with a blood-vessel), in the massive gland the individual gland cell presents all its surfaces towards the intercellular lumina penetrating the mass of the gland, *i.e.* the intercellular lumina, according to Reis, surround each cell. Reis suggests that this supposed feature is for the purpose of facilitating the exit of the gas through the gland into the bladder. As Oppel justly remarks, how can a cell thus shut off from all vascular tissue obtain nourishment? So far as my observations go, there is no reason to suppose that a single cell of the massive gland is not in contact with a capillary. I cannot, however, agree with Oppel's groundless suggestion that tubular and massive glands possess different functions, tubular glands being secretory and massive absorptive. If this be so, from whence do fish with massive glands obtain their bladder oxygen?

† Mr. Tate Regan informs me that, according to Holt, "*Coris giofredi*" is merely the female form of *C. julis*.

about twelve cells deep, is an example of the massive type possessing a squamous lining epithelium. The lumina consist of small crack-like spaces visible here and there between the cells (largely arranged in vertical rows) in transverse sections of the gland, and they form a system of fine anastomosing channels opening at various points on the surface into the bladder lumen. Gas bubbles were occasionally to be found in the gland cells. I did not observe any intracellular capillaries, but they doubtless occur; intercellular capillaries, on the other hand, are extremely plentiful and occur largely near the extreme edge of the gland underlying the layer of flattened cells. The glandular epithelium of *Coris julis* differs from preceding examples of the massive type in that the cells lying next the bladder cavity form the squamous layer, or in places layers, just mentioned.

It was doubtless because Corning (23) chiefly examined "red bodies" of this extreme massive type, as *e. g.* that of the Pike, that he concluded that the bladder glands possess no lumina, and, indeed, were it not for our knowledge that most bladder glands possess lumina and that these vary greatly in volume according to the state of activity of the gland, we should probably come to the conclusion if studying exclusively the same material.

CORVINA NIGRA Cuv., SARGUS RONDELETHI C. V., BALISTES
CAPRISCUS L., CARANX TRACHURUS and SERRANUS CABRILLA L.

The "red bodies" of these genera all belong to the massive epithelium type, although they vary in appearance macroscopically (Pls. VII. & VIII. figs. 59-61) in correspondence with the varying configuration of the rete mirabile. The "red body" of *Balistes* alone is situated near the *posterior* end of the ventral wall of the bladder. In all cases the glandular epithelium has the usual structure—a thick mass of cells, penetrated by large or small intercellular and intracellular lumina and capillaries.

ZEUS FABER L.

The "red body" of *Zeus faber* is of the curious form depicted in figure 62 (Pl. VIII.), and occupies the anterior half of the ventral surface of the bladder wall. It has already been figured by Vincent & Barnes (75). The "red body" consists of two lateral divisions, each division being shaped like a **C** divided into three parts, and its concavity facing that of its companion. The primary artery and vein enter at about the centre of the "red body" and supply branches to the retia mirabilia lying on the inner sides of the gas gland thickenings. Anteriorly to the "red body" a thick median longitudinal muscle-band is developed in the ventral bladder wall. The glandular epithelium of the John Dory is of the ordinary massive type, though more folded in appearance than is usual. It is significant that Vincent & Barnes, who wished to reduce all "red body" glandular epithelia to the folded type, remark

that "we have not been able to obtain sections which show the direct transition to the tubular glands, but there is no doubt that the epithelium takes on fairly suddenly a markedly glandular aspect."

A conspicuous feature in the gas gland of *Zeus* is the presence, among cells of the ordinary size, of giant cells and nuclei six to ten times as large (Pl. IX, fig. 65). Similar giant cells also occur in *Ophidium barbatum*, *Pierasfer acus*, *Peristethus cataphractus*, *Balistes capriscus*, *Trigla gurnardus*, and, in some instances, in *Gobius paganellus* and in other genera. The only suggestion concerning the origin of these giant cells with which I am acquainted is that implied by Deimeka (29), who states that the similar giant cells in *Perca* give rise by *amitotic division of the nucleus* to groups of cells of the usual size—the implication being that the giant cells are primary products of development and cells of normal size secondary products. My observations have led me to a somewhat different conclusion. In the first place, in many gas glands containing giant cells (*Zeus*, e.g.), these giant cells usually lie more or less remote from the region where the blood-vessels enter the gland—giant cells are nearly always found towards the periphery or edge of the gland epithelium; secondly, there is every gradation in size from the smallest to the giant gas gland cells, the smallest always being situated in those portions of the gland next the large blood vessels, *i. e.* at the bases of the folds of folded glands and at the "hub" or point of entry of the blood-vessels in massive glands (see figs. 46, 49, 52, on Plates V., VI., VII. *e.g.*) in addition to other regions, the cells of intermediate size like the smallest cells being found in most regions of the gland and the giant cells, as already mentioned, towards the peripheral portions of the gland*; thirdly, among the smallest cells mitotic figures are quite common in many of my preparations, but among the intermediate and giant cells it is rare to find mitosis, though I have seen several cases of it in intermediate cells and once or twice even in cells approaching giant size; finally, I can confirm Deimeka in his statement that the great majority of giant cells and many intermediate cells divide amitotically. Figure 63 (Pl. VIII.) shows the appearance of the epithelium at the base of the gland of *Zeus*, where the capillaries of the rete enter. It will be observed that in this genus many of the basal portions of the massive epithelium are syncytial in character, no cell-outlines being distinguishable, and that occasionally mitotic figures are present among the nuclei of these syncytia. As we proceed from these syncytial masses towards the periphery of the gland, cell-outlines soon become more and more distinct and larger in size, but, as we might anticipate, though the giant cells are usually found towards the periphery of the gland, yet giant nuclei and nuclei of a size intermediate between these and the smallest nuclei are to be found

* Reis remarks that in *Sargus* (in which there are no giant cells) the cells are largest next the blood-vessels and decrease in size towards the periphery, where they are squamous in character. This is not the case in my preparations of *Sargus rondeletii*, where the cells are practically uniform in size at all depths of the gland, except perhaps at the very edge, where they are squamous.

in the syncytial masses at the base of the gland. In cells of intermediate size and in giant cells amitosis occurs, as I have already stated. Figure 64 illustrates several phases of direct division, but I must also mention that the huge nuclei of many giant cells, like those of myeloplaxes of bone-marrow, appear sometimes to divide into several—three, four, or more—nuclear lumps at a single division, though I do not remember to have seen cells containing more than two nuclei in the resting condition. These various facts considered collectively seem to suggest that in cases like *Zeus* the initial unilaminar epithelium of the bladder wall must contain nuclei of various sizes and that the nuclei of each size, associated with cytoplasm, multiply in order to produce the massive gland of the adult fish. As the nuclei become more numerous they migrate from the centre of proliferation, the small nuclei occupying cytoplasm which, owing to the size of the nuclei, becomes subdivided up into small areas, the larger nuclei, fewer in number, also occupying cytoplasm which, owing to the superior size of the nuclei and their slow rate of division, remains to a large extent unsubdivided, so forming the giant cells round the giant nuclei. There is no evidence in my preparations of giant cells splitting up into groups of small cells. The small cells multiply by mitotic division, and amitosis is only found in the case of the larger nuclei—those of the giant cells and larger intermediate cells. As to the origin of the giant nuclei I can give no information. It is stated that the cells of bone-marrow and some glands divide mitotically after undergoing amitosis, so that there is no theoretical objection to supposing that the small syncytial nuclei have been produced, as Deineka supposes, by amitotic subdivision of giant nuclei, but I have never seen any appearance of this taking place and I very much doubt its occurrence: nearly all the appearances of amitosis which I have observed have been in connection with the nuclei of giant cells, *i. e.* far away from the syncytia. As to the reason for amitosis occurring in connection with giant nuclei, it is only possible to readvance the old suggestion that it is a prelude to degeneration. The gas gland is in *Zeus* and presumably other types constantly being regenerated at its base, and therefore there is ground for supposing that cells at the periphery of the gland become used up and degeneration is the natural result of this. But this supposed degeneration of the cells has nothing to do with the violent decomposition of cell-substance assumed to occur by Nusbaum & Reis in connection with the production of gas—a phenomenon for the existence of which I can find no evidence and in which I quite disbelieve.

GADUS MORRHUA.

The "red body" of the Cod* is known to every student of comparative anatomy as a large red mass situated anteriorly in the bladder. As Vincent & Barnes (75) say, the free surface of the "red gland" shows "many small clubbed processes, packed

* I used common "Codling" caught off Deal.

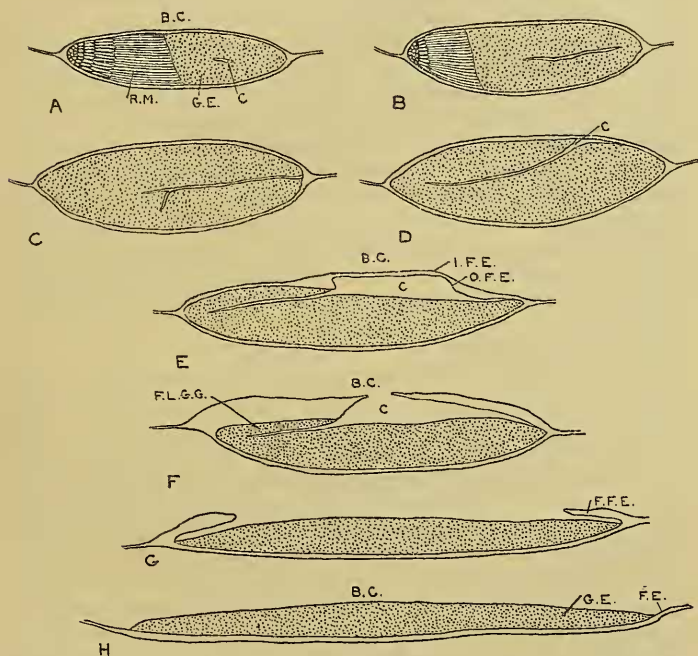
tightly together, each consisting of a red mass of tissue, surmounted by a cap of yellowish mucilaginous-looking material." Each club-shaped process "consists essentially of two parts:— (1) a mass of capillary blood-vessels, lying side by side, conveying blood to and taking blood from the second parts; (2) a glandular portion." In figure 66 (Pl. VIII.) is represented diagrammatically a transverse section of the gland of the "Codling," which shows the "clubbed processes" well in section, also one "cap of mucilaginous-looking" connective tissue. Vincent & Barnes go on to say that "when the capillaries arrive at a point about .2 mm. from the free surface of the gland, they interdigitate with involutions of the glandular epithelium, which dips down between them and forms a covering for them, thus constituting tubular glands .2 mm. in length." In other words, Vincent & Barnes state that the glandular epithelium of *Gadus* is that which I have described above for the Eel and *Syngnathus*, and they provide a diagram of what they suppose to be the folded arrangement of the epithelium. I am quite willing to admit that the small columnar cells which compose the glandular epithelium are in general arranged round strands of vascular connective tissue, and that occasionally the intercellular lumen is so extensive and so disposed as to give the glandular tissue an almost folded appearance, but there is no real ground for comparing the massive structure of the glandular epithelium of the "Codling" to the folded epithelium of *Syngnathus*. The gas gland of the "Codling" is of the massive type, in which the intercellular lumina are very abundant and occasionally very large. The capillaries are very small and run in the numerous thick strands of connective tissue. As Vincent & Barnes mention, caps of connective tissue, continuous with the connective tissue penetrating the glandular epithelium, are often present. I have not examined them in detail.

CEPOLA RUBESCENS L.

The "red body" of *Cepola rubescens* is situated anteriorly in the ventral wall of the bladder and has somewhat the shape of a bell (Pl. VIII. fig. 67), the handle being formed by the rete mirabile. The artery and vein enter the "red body" anteriorly at the top of the bell-handle, and there give rise to the rete mirabile in the usual way. The rete mirabile gradually diminishes as it supplies the mass of the gas gland, which first appears, in the series of transverse sections, on the right side of the "red body" (text-fig. 59, *G.E.*). The peculiar feature about the "red body" of *Cepola* is the curious folding of the glandular mass anteriorly. The gas gland extends a considerable way posteriorly, as seen in figure 67, and the best way to comprehend the folding anteriorly—the folding, that is to say, of the whole mass of the gas gland, not the folding of the epithelium hitherto described—is to observe the diagrams of the sections contained in text-figure 59 in the reverse order, *i. e.* observe H first and A last. Diagram H represents the glandular epithelium (the dotted area *G.E.*) as a mass of cells developed from the general squamous epithelium (*F.E.*) lining the

bladder cavity (*B.C.*) in the median line of the ventral wall of the bladder. In diagram G it is to be observed that the squamous epithelium on each side of the glandular mass has become folded (*F.F.E.*) and projects into the bladder cavity towards the median line. In diagram F these folds have nearly met in the middle line and lie dorsal to the gas gland in the bladder cavity; also it will be observed that on the left side the glandular mass itself has become completely reflected towards the median line (*F.L.G.G.*), and lies in close apposition with the rest of the glandular mass. Still more anteriorly, the two lateral folds of squamous epithelium have, as shown in diagram E, united in the median line, so that

Text-fig. 59.



Diagrammatic transverse sections through the anterior end of fig. 67 (Pl. VIII.), where the rete mirabile supplies the epithelium of the gas gland. The diagram is fully explained in the text.

two separate epithelia now lie in the bladder cavity above the gas gland, the upper of which (*I.F.E.*) is continuous with the squamous epithelium lining the rest of the bladder wall, and the lower (*O.F.E.*) is continuous with the two sides (the left reflected as just described) of the glandular mass, which latter therefore now forms the thickened ventral wall of a tube. Diagram D shows that the dorsal thin wall of the tube just mentioned is being replaced by extensions of the glandular mass, this replacement of the thin epithelium being chiefly due to the increase in size of

reflected left side of the glandular mass. In diagram C the reflected left side of the gas gland has increased so as to extend over the opposite or right side of the gland, a narrow crack (continuous posteriorly with the bladder cavity) only being left between the dorsal and ventral halves of the gas gland. In diagrams B and A, owing to the coalescence of the glandular masses of the dorsal and ventral portions of the gas gland on the right side (*i. e.* the replacement of the short strip of thin epithelium which in C closed the tube on the right side), the narrow crack (C) becomes bounded on all sides by the glandular substance, and this crack narrows anteriorly until it disappears shortly before the gas gland itself terminates.

The mass of glandular epithelium (Pl. IX. fig. 68) resembles that of *Coris julis* in being (apparently) almost devoid of gland lumina. Small intercellular cracks are visible here and there between the cells in my preparations, but that is all to be observed in the inactive gland. On the other hand, the glandular mass is penetrated by capillaries, and the large cells appear to arrange themselves round these capillaries to some extent, giving the gland in section almost a lobulate appearance. The cytoplasm of the cells is conspicuously darkened where in contact with the capillaries. Now and again large cells are found with a very large nucleus, but these are rare. Finally, it may be mentioned that the capillaries of the rete mirabile possess the peculiar endothelium which I have already described in *Gobius paganellus* and found in most "red bodies."

PERCA FLUVIATILIS.

The "red body" of the freshwater Perch is a diffuse organ situated anteriorly on the ventral and lateral portions of the bladder wall (Pl. IX. fig. 69). The retia mirabilia are seen as small fan-shaped tufts present at the extremities of the various branchings of the closely-associated artery and vein (both included in the red streak seen in the bladder wall), and bordering these tufts of rete are the areas of gas gland. The gas gland is of the ordinary massive type, but feebly developed compared with the gas glands of most marine fish, consisting in its thickest parts of not more than six cell-layers (see text-fig. 62, p. 240) and dwindling to one at its edges (Pl. IX. fig. 70). The giant cells described by Deineka in young Perch are only of very moderate size in my preparations*, indeed, not so large as those I have figured in the case of *Zeus*; as in *Zeus*, amitosis occurs in connection with most of the cells. With reference to the suggestion of Deineka that these giant cells subdivide into groups of the smaller cells as a consequence of the fragmentation of the nucleus, I have already advanced reasons for regarding this as improbable (see *Zeus*).

Previous Descriptions of the Structure of the Ophidium or Third Type of "Red Body."

I shall here merely enumerate the chief histological descriptions of gas glands of the massive type. Emery 1880 (33), *Fierasfer*;

* My Perch were from 10-14 cm. in length.

Coggi 1886 (25), *Serranus*, *Perca*, *Balistes*, *Corvina*, *Julis*, *Motella* and other types, and 1889 (23) *Esox lucius*; Corning 1888 (28), *Esox*, *Perca* and other types; Vincent & Barnes 1896 (75), *Zeus*, *Gadus* and other types; de Seabra 1897 (31), *Esox* and others; Jaeger 1903 (44), *Sciæna*, *Lucioperca*; Deineka 1904 (29), *Perca*, *Esox* and other types; Bykowski & Nusbaum 1904 (24), *Fierasfer*, *Ophidium*; Reis & Nusbaum 1905, 1906 (62, 63), *Polyacanthus* (*Macropodus*), *Fierasfer*, *Ophidium*, *Charax*, *Perca*; Reis 1906 (64, 65), *Sargus*, *Charax*, *Pagellus*, *Corvina*, *Umbrina*, *Chrysophrys* and others described in a general way.

*Summary of Part I. and General Conclusions respecting
Gas Gland Structure.*

From the foregoing it is now manifest that all teleostean "red bodies," i. e. the vasculo-glandular gas-producing organs situated in the bladder wall, are composite structures consisting of two distinct and sometimes separate parts—the *glandular epithelium* or *gas gland** and the *rete mirabile*†. The mutual relationships of these two parts are quite constant, and though the gas gland must be described as the essential part, yet the rete, on account of its constant‡ presence and undoubtedly highly important function, is probably of almost equal importance. The rete mirabile bipolare geminum (Müller, 53), i. e. the arterial and venous rete already described, is always formed in connection with the artery and vein supplying the gas gland, and may either be anatomically quite distinct from the glandular epithelium (Eel type), the arterial and venous capillaries reuniting to a varying extent on the gland side of the rete to form large arteries and veins before supplying the glandular epithelium, or the rete mirabile may be contiguous with the glandular epithelium, the capillaries of the rete undergoing little (e. g. *Syngnathus*) or no (e. g. *Ophidium*) reunion before supplying this.

The diffuse pancreas, when present, is often intermingled with the arteries and veins situated at the extreme anterior end of the rete, and so actually becomes situated inside the bladder wall; it was at first, on account of several peculiarities, mistaken by me for a new gland with a special function connected with the gas gland, but this view was quite a mistaken one, the penetration of the pancreas into the bladder wall being of no physiological significance.

The gross anatomical differences, often visible to the naked eye, which are to be found in "red bodies" taken from different fishes, are determined by the disposition and degree of subdivision of the rete mirabile—since the distribution of the glandular

* The "drüsige säume" of Müller and the "corpo epitheliale" of Coggi.

† The "vaso-ganglion" of Owen and "organo vascolare" of Emery.

‡ That is to say, using the term "gas gland" in the usual sense as equivalent to "oxygen gland" (see Part II.), the rete is constantly associated with it; the rete, however, is absent in the cases of those bladders the lining-epithelia of which only, or principally, "secrete" nitrogen and carbon dioxide.

epithelium must evidently correspond with that of the rete mirabile. It need hardly be pointed out that the gross anatomical simplicity or complexity of the "red body" by no means always corresponds with the histological simplicity or complexity of the gland epithelium, and that this latter affords the only true basis for a classification of "red bodies." One of the most simple (though not the most primitive) conditions of the rete mirabile is when it is single and compact*. In this condition it may be separate from the glandular epithelium and ovoid in shape (*Ophichthys imberbis*, fig. 9, *e. g.*), or contiguous with the glandular epithelium and circular in form (*Gobius*, fig. 22, *e. g.*), conical (*Cepola*, fig. 67, *e. g.*) or fan-shaped (*Ophidium*, fig. 48, *e. g.*), &c. In some cases the rete mirabile is divided into two, and here these are separate from the glandular epithelium and ovoid in form (*Anguilla*, text-fig. 53, *Myrus*, *Muraena*, *Ophichthys serpens*, &c.). In other cases the rete mirabile is divided up into several distinct parts, which often have a radiate arrangement: *e. g.* in *Peristethus* the rete is split up into some ten radiating strands (fig. 37 and text-fig. 57) and in some species of *Gobius* into seven or more. In most cases, however, the initial artery and vein supplying the bladder give off numerous arteries and veins in pairs (an artery and a vein to each pair, of course) at intervals so as to give rise either (*a*) to continuous sheets of rete mirabile, so to speak, very often two in number, one on each side, as in *Corvina nigra* (fig. 59), *Sargus rondeletii* (fig. 60), and *Smaris vulgaris* (fig. 45), or (*b*) to sheets divided up in variable degrees, as *e. g.* in *Box boops*, where the sheets are divided into outer and inner on each side (figs. 51, 52), or in *Zeus faber*, where there are three separate sheets—anterior, median, and posterior—on each side (fig. 62), or (*c*) to numerous separate small strands or tufts of rete mirabile, each strand supplying a small area of glandular epithelium: thus, in *Coris julis* (figs. 56, 57) the rete is seen under the microscope to be subdivided into many small strands supplying the small area occupied by the gas gland; in *Trigla hirundo* also a similar condition exists, but here, indeed, the twigs extend nearly over the whole interior of the bladder cavity (fig. 43) instead of being restricted to a small area; in other cases where the tufts are larger and more separated, these can be seen with the naked eye, as, *e. g.*, in *Atherina hepsetus* (fig. 53) and *Perca* (fig. 69) and in the well-known case of the Cod (fig. 66), in which the subdivision of the rete mirabile reaches its maximum.

Whether the condition of the rete mirabile with which we started, *i. e.* as a single large well-defined body, separate from the

* Reis (64) supposes that the horseshoe-shaped gland of *Ophidium*, *e. g.* (see my fig. 48 and compare it with figs. 59, 60, and 62), is the form of gas gland and rete from which all others are derivable, but it seems to me that the simple oval rete of *Syngnathus* in connection with an extensive area of glandular epithelium, is still more simple; the most primitive condition of the epithelium and associated blood vessels is, of course, that of many freshwater teleosts in which practically the whole of the internal unilaminar living-epithelium of the bladder constitutes the gas gland, and the arterial and venous capillaries have not become associated to form a rete. In general it may be said that the more diffuse the rete and gland the more primitive their nature.

glandular epithelium (*Ophichthys imberbis*, e. g.), is more primitive or more evolved than the condition which we found in the Cod, e. g., is a question I shall not discuss; I will merely say that there are several reasons for accepting, with de Seabra (31), the latter view. The fact that *Ophichthys* and *Anguilla* possess a very simple type of glandular epithelium is no objection to this view, since the rete of *Cepola* and other genera possessing complex types of epithelium is almost as simple.

The glandular epithelium, as we have seen, may line the whole interior of the bladder (as in many Anguilliformes and freshwater teleosts) or be restricted to a small area of the internal surface (*Ophidium* type). The *Syngnathus* type is more or less intermediate between these two conditions.

The character of the glandular epithelium varies greatly. The cells composing it may be arranged to form (a) a single unfolded layer (*Myrus*, e. g.); (b) a layer thrown into simple or complex folds, so that channels are formed leading from the surface of the glandular epithelium to the bladder lumen (*Anguilla*, *Gobius*, *Syngnathus*, *Trigla*, *Smaris*); (c) a thick mass in which the deeper cells can only communicate with the bladder lumen by a system of large or small anastomosing channels (the intercellular lumina) which penetrate the cell-mass and open into the bladder cavity by small apertures situated on the internal surface of the glandular mass (*Ophidium*, *Coris*, e. g.). At present neither embryology nor comparative anatomy provides evidence to justify the inference that this massive type of gland epithelium is in all cases derived from the folded condition—the folds on this view being supposed to have become elongated, laterally branched perhaps, closely apposed and fused at intervals to produce the cell-mass penetrated by narrow anastomosing channels. It would gratify me to be able to agree with Reis (64) who states that she is able to trace a complete developmental series of complications in the structure of the gas gland epithelium, the simple unfolded cell-layer forming the starting and the cell mass of *Coris*, *Cepola*, or *Esox* the culminating points of the series; but, as I have already stated, the available embryological and anatomical evidence suggests that the two complex types of folded and massive gas gland epithelia were separately derived from the primitive layer of glandular cells by folding (i. e. the plane of cell-cleavage is always at right angles to the plane of the bladder wall) and by thickening (the plane of cell-cleavage being parallel with the plane of the bladder wall) respectively. It is true that forms of gas gland epithelia occur apparently transitional in character between the folded and massive types (*Trigla gurnardus*, *Smaris maurii*, *S. vulgaris*), but I have supplied reasons for regarding these as glandular epithelia of the folded type which have become secondarily massive in form and so differing from the truly massive glands which never pass through a folded stage in their development. There is no reason, however, why certain glands should not be truly intermediate in structure, i. e. in which the cells during development divide simultaneously in planes both

perpendicular to and parallel with the plane of the bladder wall, but I know of no examples of such. Subsequent embryological enquiry can alone prove which of these views is the correct one.

The minute structure of the cells of the gas gland epithelium is doubtless constant throughout the entire series of "red bodies." Gas bubbles are in all cases generated in the interior of the cells (usually in the neighbourhood of the nucleus) when the glands are active, and these bubbles are ejected by the cells into the gland ducts and bladder cavity where they explode, the shattered walls of these bubbles giving rise to the masses of granular matter already described. Vincent & Barnes analysed this granular matter (which, as before mentioned, must be carefully distinguished from the disintegration products of the red blood corpuscles alone found in the blood-vessels) of the Cod and found it to consist principally of a nucleo-proteid; and since the cytoplasm which forms the wall of the gas bubble is also composed for the greater part of this substance, the result of this single analysis is confirmatory of the view just expressed, as is also the fact that a large amount of this granular matter is always associated with the presence of numerous gas bubbles. The intracellular ducts, which, with the other cytological features of the gas gland, have been so well figured by Bykowski & Nusbaum (24) and Reis & Nusbaum (62) within the last few years, are doubtless also constant features of active teleost gas glands, though I am of opinion that some of these intracellular channels are due to the expulsion of bubbles from the cells and not purely nutritive as Reis & Nusbaum suppose. The intracellular capillaries, on the other hand, are permanent structures, and the fact that they occur so rarely as compared with the enormous number of these structures found in connection with the liver cells can only be explained by the relative inactivity of the teleost gas gland.

I have already described and figured the hæmolytic disintegration of the red blood corpuscles (erythrocytolysis as it may be called) which occurs in the blood-vessels associated with the active gas gland of *Syngnathus acus*, and this same phenomenon is to be found in connection with other of my preparations of active gas glands and is doubtless a result of the gland's activity. In this process of hæmolysis many of the erythrocytes become entirely broken up, first into large globules and finally into the granular matter which is present in such large quantity in the capillary lumina, whilst others are distorted in shape and only partially broken up, looking very much as if influenced by some toxic substance, as Jæger suggests (see Part II.). This condition of the erythrocytes cannot be due to imperfect fixation since great care was exercised in the fixation and preservation of these gas glands; moreover sections through other vertebrates—Elasmobranchs *e. g.*—fixed by similar methods, exhibit the blood vessels as practically free from granular matter (the small amount sometimes present simply representing coagulated plasma) and the erythrocytes as retaining their form.

Another fact which I wish to lay stress upon, especially in view

of the theory advanced in Part II., is the distribution of the granular matter in the capillaries of the rete mirabile. Careful examination of my sections* of *Gobius niger*, *Gobius minutus*, *Syngnathus acus*, *Peristethus cataphractus*, *Gasterosteus spinachia*, and some others, in all of which the gas glands are more or less active, had led me to the conclusions that granular matter is present in the venous capillaries of the rete to a far greater extent than in the arterial, and that the relatively small amount of granular matter present in the arterial capillaries is chiefly situated next the distal pole of the rete—the pole next the gas gland. The small calibre of the arteries compared with the veins and the few series of sections of active glands, well fixed and well stained, which I possess render it difficult to be absolutely certain of the above conclusions, but I am as certain as the limitations of my material will permit me to be. The bearing of these facts on the theory already outlined on page 198 is perhaps already apparent, but I shall reserve discussion for Part II. (see p. 232).

As already implied, teleost gas glands are best classified according to the character of the glandular epithelium. We divide gas glands into two groups:—(1) gas glands in which the glandular epithelium is a single layer, which may either remain unfolded or become folded in variable degrees; and (2) gas glands in which the glandular epithelium is many-layered, the mass of cells being penetrated by fine ducts. With the exception of a few cases (*Trigla gurnardus*, *Smaris maurii*, *S. vulgaris*, and some others) in which the much-folded epithelium has secondarily assumed a massive character, this massive type of epithelium is probably not derivable from the folded type. Also in this second group of gas glands the rete mirabile is always contiguous with the glandular epithelium, reunion of the capillaries never occurring (*Ophidium*, *Fierasfer*, *Box*, *Atherina*, *Coris*, *Balistes*, *Sargus*, *Caranx*, *Charax*, *Serranus*, *Zeus*, *Gadus*, *Cepola*, *Esoc*, *Perca*, &c.).

Group 1 is subdivisible into two minor groups:—

- (a) The rete mirabile of the gas gland consists of either one or two bodies distinctly separate from the glandular epithelium, the capillaries of the rete reuniting to form large arteries and veins before they come into connection with the glandular epithelium (*Anguilla*, *Myrus*, *Ophichthys*, *Muraena*, &c.).
- (b) The rete mirabile of the gas gland is contiguous with the glandular epithelium, although a small amount of reunion of the capillaries occasionally occurs (*Syngnathus*, *Gobius*, *Siphonostoma*, *Nerophis*, *Gasterosteus*, *Peristethus*, *Trigla*, &c.).

In concluding this account of the practical work connected with

* Observations of the rete mirabile, when sectioned longitudinally, are preferable from this standpoint; the arterioles are, of course, distinguishable from the venules by thicker walls, by small calibre, and by the peculiar endothelium before mentioned

the present paper, I wish to express my great indebtedness to the Council of King's College, London, for generously allotting me the sum of thirty pounds out of the Government grant to the College for 1907 to defray a part of the expenses incurred by me during my visit to the Naples Zoological Station in April of that year. I wish also to express my indebtedness to the Naples Zoological Station Table Committee of the British Association for the occupancy of the table at Naples during the three weeks just referred to, and to the staff of the Naples Zoological Station for the large amount of trouble they took in supplying me with the material I required in connection with this and other subjects. Further, I wish to thank Professor A. Dendy, F.R.S., and Professor S. J. Hickson, F.R.S., for kind recommendations in connection with the above-mentioned grant and occupancy of the Naples table respectively, Professor J. P. Hill, D.Sc., and Dr. G. C. Chubb for recent friendly assistance, Mr. C. Tate Regan, M.A., for kindly advising me in connection with the systematic nomenclature adopted in this paper (the nomenclature of Günther's Catalogue), and Mr. C. Biddulph, who has considerably lightened the burden of preparing some hundreds of slides. Finally, I am also much indebted to the Council of the Royal Microscopical Society for kind permission to occupy their table at the Plymouth Marine Biological Laboratory during a week in August, 1909.

PART II.—A FEW SUGGESTIONS CONCERNING THE PHYSIOLOGY OF THE "RED BODIES."

It was my original intention to include in Part II. of this paper a *résumé* of our present knowledge of the physiology of the gas bladder*, but although, with this end in view, I have become thoroughly acquainted with the whole of the vast literature dealing with the subject, yet other work prohibits me at present from thus devoting the time necessary for the composition of such a review. This being the case, I shall content myself with providing an outline sketch of the chief views which have hitherto been advanced to explain the mode of working of the "red bodies," in order that the reader may be in a position to estimate the value of the few additional suggestions I have to make.

The several views which have been held concerning the origin of the three† principal gases—oxygen, nitrogen and carbon dioxide—contained in variable proportions in the gas-bladder cavity, can be classified into two categories: (1) the view (first attributed to Redi) that these gases are derived directly from the atmosphere, and (2) the views (lineal descendants of Needham's secretion theory‡) that these gases are derived more or less

* A recent account of the functions of the bladder will be found in Baglioni (18).

† Argon is also stated to occur in the bladder.

‡ It should be noted that authors in discussing the production of gas employ the term "secrete" in a very loose manner, some thereby meaning a true process of secretion, such as that which occurs in the sebaceous gland (Nusbaum & Reis, *et. g.*), others (Hüfner, Jaeger) merely meaning a process of pumping from the blood.

directly from the blood stream. The first view, abandoned by nearly all modern authors, is, however, persistently maintained by Thilo (70, 71, 72). Thilo's principal contention, stated briefly, is that the blood of a fish is not sufficient in quantity to contain the amount of gas found in the bladder, and that even if this were the case the circulation of the fish is so feeble that the blood could not replenish an emptied bladder in the time experiment proves that it can be replenished. He therefore asserts that in all cases the bladder-gas must have been procured by the fish directly from the atmosphere: Physostomi can always renew their bladder-gas by rising to the surface of the water and passing air along the ductus pneumaticus, whilst Physoclisti are apparently under the hard necessity of absorbing a sufficient supply when young, and therefore before the duct has degenerated, to last them throughout life, though Thilo further maintains that in many cases even adult Physoclisti are still able to procure fresh supplies by passing air, either swallowed from the atmosphere or extracted in some inexplicable manner from the water, along the strand of tissue representing the vestigial duct. Thilo performed experiments and, according to his statements, obtained results which strongly support his view. He asserts, *e. g.* that he cut off the vascular supply of the previously-emptied bladder in *Tinca* (a physostome) and that in thirty hours it became refilled with gas. Seeing that the blood-supply was absent, he argues that this fresh supply of gas must have been obtained from the atmosphere by passage through the pneumatic duct. I, however, cannot find that he analysed the gas produced under these conditions, and, since atmospheric air and secreted gas nearly always consist of oxygen, nitrogen and carbon dioxide associated in very different proportions in the two cases, his contention as to the source of the gas receives very little real support from his experiments. A few of the many obvious objections to Thilo's hypothesis may be stated. In the first place, the percentage composition of the three gases contained in the bladder is, as just mentioned, quite different from that present in the atmosphere, and in the cases of oxygen and nitrogen these gases are often present in such quantities as to exert a pressure many times greater than the pressures they exert in air—both of which elementary facts are fatal to Thilo's view. Thilo's contention that the blood of a fish with its feeble circulation is incapable of providing the quantity of gas required by the bladder of course begs the question at issue, and he might with equal force contend that the tissues of a siliceous sponge, for example, must be incompetent to deposit a massive siliceous skeleton, seeing that 100,000 parts of sea-water contain little more than one part of silica in solution and that diatoms and other minute organisms are serious competitors even for this small quantity (Sollas). Further, Thilo's experimental results are not in accordance with those obtained by Hüfner (38), Jaeger (45), and others, and, indeed, Thilo's own experiments failed to render his contention even probable since the gland cells of the bladder would not necessarily cease their activity immediately a large

part of their blood-supply was cut off. It is also certain that most physostomous fishes do not obtain their gases from the atmosphere *via* the pneumatic duct, since the pneumatic duct is now known to be merely one of the two principal mechanisms (the other being the "oval") employed by fish for the elimination and not the obtainment of gas. Finally, Thilo's theory fails to account both for the degeneration of the ductus pneumaticus in Physoclisti (since, according to Thilo, it is still functional) and for the presence of the complex "red bodies" which are the very structures needed by the fish on account of the feeble blood-circulation referred to by Thilo.

Theories which derive the bladder gases from the blood stream can again be grouped into two classes, viz. those which suppose that the blood gives up its gaseous constituents more or less directly to the bladder, the gas passing straight from the capillaries of the rete mirabile, or wall of the bladder, into the bladder lumen, and those which regard the gas gland as the special organ which extracts the gas from the blood. The first class of opinions, to some extent associated with the name of Moreau, is now quite out of date, though still to be found stated in some recent text-books. The second class of opinions comprises two quite distinct views as to the exact function of the gas gland—two views which at the present time are held with equal tenacity by the schools represented by Jaeger and Nussbaum & Reis respectively. I shall first state briefly the view of Jaeger (44-48).

Jaeger, following Hufner (38), holds that the gas gland is primarily a pumping apparatus, that is to say, an apparatus for pumping the gases contained in the blood into the bladder cavity. The pressure exerted by the gases in the blood is, of course, considerably less than that exerted by the gases in the bladder*, and it is the function of the gas gland to force the gas from the blood into the bladder lumen against this superior pressure. Jaeger further supposes that the disintegration of a certain percentage of red blood corpuscles is effected by the secretion on the part of the gas gland cells of a toxin which is poured into the blood for this purpose. The object of so breaking up the erythrocytes is to enable the gas gland cells, in some way not described, to lay hold of, with greater facility, the oxygen thus scattered in the corpuscle fragments. Indeed, Jaeger, following Moreau, regards the gas gland as a mechanism essentially concerned with the pumping of oxygen—as an oxygen gland, in short,—a view confirmed both by the great development of this gland and by the disintegration of the erythrocytes in connection with bladders containing a large percentage of oxygen, and also by the contrary fact that in the bladders of Cyprinoids and many other freshwater fish which mostly contain nitrogen, the gas glands are absent—the ordinary squamous epithelial lining here being capable, without undergoing any special modification into a

* E. g. Jaeger (46) states that in deep-sea fish the partial pressure of the oxygen in the blood only amounts to about one-fifth of an atmosphere, whereas the oxygen in the bladder may possess a partial pressure of over forty atmospheres.

gland, of pumping in the nitrogen alone required. The reason for the special development of the oxygen gland in many deep-water fishes* is because oxygen alone among the gases present in the bladder is capable (in virtue of its property of combining in large quantity with the hæmoglobin of the blood) of being either rapidly produced (by the gas gland) or rapidly absorbed (by the "oval"†), and this rapidity of production and absorption is essential in the case of fishes which undergo considerable changes of pressure in the bladder. Deep-water marine or freshwater fish differ from most freshwater fish in that living in great depths of water those possessing migratory habits in a vertical direction require apparatus for adapting the volume of gas in the bladder to the violent changes of pressure experienced; most freshwater fish, on the other hand, live in comparatively shallow water—in inland lakes and rivers—and thus lead placid lives, experiencing little or no changes of pressure in the bladder, and for these fish it is evident that there is no need for the special development of an oxygen gland, which makes extravagant use of the gas which the fish requires for respiration: nitrogen and carbon dioxide suffice‡. With reference to the spherical spaces present in the cells of the gas gland, Jaeger altogether denies that they represent gas bubbles: they are merely vacuoles such as are to be found in liver-cells, *e. g.* and have no connection with the production of gas. Jaeger himself, however, describes gas bubbles as being present in the ducts of the gas gland. Jaeger also refuses to admit that the large amount of granular matter found outside active glands in the bladder lumen is the product of the gas gland cells: in short, represents the broken-down walls of exploded gas bubbles. I shall discuss these features of Jaeger's theory later.

Nusbaum & Reis (54, 55, 62–66), relying upon their extensive investigations of the cytology of the gas gland, formulate certain peculiar views§ as to the exact mode of function of the gas gland. Jaeger, as already stated, holds with Hüfner that the gas gland is essentially a pumping apparatus, though he confesses entire ignorance as to exactly how the individual gas-producing cell

* Only those deep-water fishes which migrate vertically and thus experience differences of pressure; fish which remain at one depth, however great, obviously have no more need for "red bodies" than have surface fish (see p. 228).

† Any sort of gas can, of course, be eliminated by the pneumatic duct.

‡ Mlle. C. M. L. Popta in her recent paper entitled "Étude sur la Vessie Aérienne des Poissons" (Ann. Sci. Nat. Zool., t. xii, 1910, pp. 1–160) comes to the conclusion that "la vessie aérienne des poissons se développe pour aider à la circulation de l'oxygène [et en général des gaz] dans le corps du poisson." I confess that this conclusion does not convey much meaning to me. I entirely fail to understand why the fish should develop an organ for the sole purpose of liberating oxygen into the blood in the same way I suppose that the liver liberates food material, and this is the only intelligible proposition which the author's conclusion conveys to me. Though the bladder undoubtedly in many cases performs this function incidentally, yet the fact that the bladder often contains other gases (nitrogen and carbon dioxide) the presence of which in the blood cannot be of the slightest advantage to the animal, shows that this storage function of the bladder cannot be its primary function. I cannot see that the author's conclusion in any way explains the different proportions in which the three principal gases occur in the bladder.

§ Unfortunately adopted in the last edition of Wiedersheim's 'Vergleichende Anatomie der Wirbelthiere,' Siebente Auflage, 1909.

extracts the gas from the blood and forces it against great pressure into the bladder: the supposition is, on his view, simply a legitimate deduction from the facts, and the details of the process, upon which he can throw no light, are of secondary importance. Nusbaum & Reis, on the other hand, profess to have discovered in the cytology of the gas gland cell, some details of the process which prove that the gas gland is not the mere pump which Jaeger assumes it to be. The view of Nusbaum & Reis is briefly this:—Examination of the cells of the gas gland proves the existence of spherical spaces in the cytoplasm which are not mere vacuoles as Jaeger assumed, but represent actual gas bubbles being formed in the cell. These gas bubbles are also to be found in the act of being ejected from the cells, and also lying freely in the gland ducts and bladder lumen, Jaeger himself necessarily agreeing that they are bubbles when found outside the cells, since liquid vacuoles could not possibly so persist. Thus much is a matter of observation, but Nusbaum & Reis contend, in explanation of these facts, that these bubbles of gas are produced by the actual decomposition of the substance of the gas gland cells, just as gases are produced by putrefaction, and in proof of this cell-decomposition they describe the actual breaking-up of the cells—the nuclei* fragmenting in the manner described by Deineka for the Perch and the cytoplasm becoming converted into the masses of granular matter found in the gland ducts and bladder lumen. The principal reason which leads Nusbaum & Reis to this surprising conclusion is the supposed incompetence of the blood to supply the percentage of nitrogen gas found in the bladder. Among minor features of this hypothesis of Nusbaum & Reis, we may mention that they regard the disintegration of the red blood corpuscles both as a source of nutrition for the gas gland cells (the granular striping of the borders of gland cells in contact with capillaries and the intracellular channels penetrating the gland-cells being evidence of the cells absorbing this nutrition) and as a source of the oxygen which, with the nutrition, is urgently required by the cells in connection with their rapid decomposition.

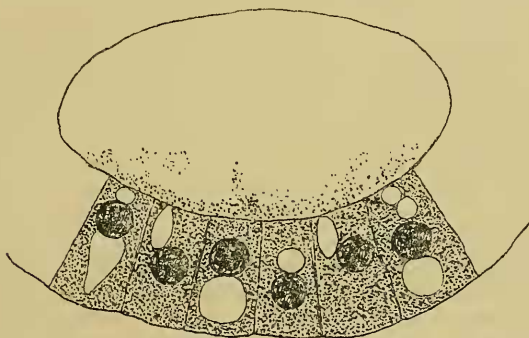
Thus the respective views of Jaeger and Nusbaum & Reis largely differ as regards the function attributed to the individual gas gland cell. Jaeger regards it as essentially a pumping mechanism; Nusbaum & Reis regard it as, primarily, mere substance to be decomposed into its gaseous and solid constituents, both being extruded into the bladder.

I will now proceed to discuss these two views as a preliminary to a statement of my own views on the subject. Apart from omissions common to both theories, which I shall endeavour to remedy shortly, the chief fault to be found with the theory of Jaeger is his refusal to recognize the origin of the gas bubbles in the cells of the gas gland. Concerning the existence of these

* I may point out in this connection that during hæmolysis the nucleus of the red corpuscles appears to persist quite unchanged, even though the cytoplasm may become entirely disintegrated.

structures there can be no doubt whatever (see Appendix B). They have been exhaustively studied by Bykowski and Nusbaum & Reis, and I have myself described and figured them in the present paper. Jaeger himself, as I have already stated, admits the presence of vacuoles in the gas gland cells, but, curiously enough, denies that they contain gas, and pronounces them similar in nature to the vacuoles in the cells of the liver. It is difficult to understand why this comparison should be made, since, from his own standpoint, it is the function of the gas gland cells to pump out gas and not to store up glycogen or fat. But even adopting his suggestion that the supposed gas bubbles are only vacuoles, we may remark that several authorities have described in the kidney "the formation of vesicles in the cells and appearances which indicate the discharge of these vesicles into the cavity of the tubules" (37); and since kidney cells, like the gas gland cells, are supposed by many modern physiologists to derive most of their excreted substance by direct abstraction from the blood, comparatively few of the constituents of urine being

Text-fig. 60 ($\times 1000$).



Vacuoles in the kidney cells of *Nerophis*, some apparently being expelled into the lumen of the tubule.

manufactured in the cell, we may certainly conclude that even liquid vacuoles can be originated and discharged in a manner precisely comparable with that of the gas bubbles, and that if they contained gas instead of liquid they would be indistinguishable. I have figured some of the vacuoles present in the kidney cells of the fish *Nerophis* (text-fig. 60). It is evident that in histological preparations no sign of the liquid urine would be visible after expulsion from the cell, whereas the gas bubble with its walls of cytoplasmic material may, like a soap bubble in air, persist for some time before bursting. Consistently with his denial of the gaseous nature of the vacuoles in the gas cells, Jaeger was compelled to regard the granular masses lying external to the gas gland in the bladder lumen merely as a bye-product of the specific activity of the gas gland.

If, however, we fully agree with Nusbaum & Reis as to the

origin of gas as bubbles in the cytoplasm of the gas gland cells, we as certainly disagree with the interpretation they put upon this phenomenon, viz. that the gas is produced as the result of the actual chemical decomposition of the cytoplasm. Like Jaeger we have found no evidence of that peculiar cell-disintegration which is supposed by Nusbaum & Reis to be the histological expression of the chemical decomposition of the cell. It is true, as Jaeger admits, that in the cells of active gas glands the cytoplasm (not the nucleus, which remains normal) often assumes a "hard-worked" appearance—the cytoplasm looks "stringy" and contains numerous empty spaces—and considering that the process of continuous pumping of gas into the bladder must be exceedingly arduous, this is not surprising. It is also true, though not admitted by Jaeger, that the expulsion of bubbles from the cell into the bladder involves, to a considerable extent, waste of cell-substance. Each bubble, as already mentioned, possesses a wall of cytoplasm, and on the bursting of the bubble in the bladder lumen or gland duct, this wall, of course, breaks down and contributes to the mass of granular matter found in the bladder lumen and gland ducts. It must be confessed, therefore, that cell-disintegration occurs to a considerable extent and is associated with the production of bubbles of gas, but this mechanical disintegration of cell-substance is quite another thing from the hypothetical chemical decomposition of cell-substance postulated by Nusbaum & Reis (see Appendix B). Jaeger criticises this hypothesis of Nusbaum & Reis in a very effectual manner. He first of all points out that it is impossible to regard the epithelium of the gas gland as analogous in its mode of working to a sebaceous gland, since the cells of the sebaceous gland decompose in order to produce a highly-complex substance chemically different from the substances supplied to them by the blood, whereas the cells of the gas gland give rise to the most simple of substances, viz. the gaseous elements oxygen and nitrogen which are supplied to them ready-made. In other words, the production of oxygen and nitrogen, unlike the secretion of the sebaceous gland, requires no elaborate cell-metabolism, and there is therefore no reason for the gas gland cell-decomposition which Nusbaum & Reis affirm*. Further, according to Nusbaum & Reis, the blood corpuscles in breaking up supply the gas gland cells with oxygen for the decomposition of their substance, from

* We may, indeed, compare the process of gas-production with another process familiar to the present writer, in which a simple substance is also supplied to the cell and again liberated by that cell though in a different form, viz. the deposition of calcareous spicules in various invertebrate groups. Calcareous spicules have, like gas bubbles, been regarded by some investigators as resulting from the actual transformation of cell-substance, but this view is not held by any modern zoologist. The spicule-secreting cell is now regarded as a mechanism for abstracting the dissolved calcareous matter from the sea-water and of redepositing it, mixed with a variable minute amount of organic substance, in a crystalline form, the aggregate of calcite crystals constituting the spicule. The work of the cell in this case is simply the abstraction and recrystallization of the dissolved calcareous salts—there is no question of cell-decomposition. The mode of working of the kidney cell may also be compared in this connection.

which decomposition oxygen is to be produced (!), but oxygen cannot be used up in decomposing the gland cells and yet be available to fill the bladder space. And as regards the supposed production of free nitrogen by the decomposition of the gas gland cells, Jaeger might have pointed out that such a fact would be unique in animal metabolism. Free nitrogen is not known to be liberated by any katabolic process. The only source of the free nitrogen found in the bladder must be the small amount of that gas dissolved in the blood plasma. But Jaeger's chief objection to the theory of Nusbaum & Reis is the impossibility of accounting for the high pressure of the gas contained in the bladder, if this gas be simply produced by the decomposition of the cell-substance. The generation of carbon dioxide from chalk at once ceases, he points out, despite a high temperature, if the gas be allowed to accumulate in the calcination chamber; and in the same way the decomposition of cytoplasm would cease long before the liberated gas could exert a fraction of the pressure usually found in the bladder*. Oppel (57), in a summary of papers by Reis & Nusbaum, also objects that whilst these authors strongly insist upon cell-degeneration yet they have not stated in what way the epithelium is regenerated after periods of activity, and I also have been unable to come to a conclusion as to the opinion held by Reis & Nusbaum on this subject. In one paper (62) these authors state in the last two sentences that they have observed mitotic figures in the gland cells, and conclude that regeneration of the gland is effected by ordinary cell-division; in a subsequent paper (63), however, they appear to embrace the curious view that the amitosis first described by Deineka in *Perca* is a result of the violent cell-decomposition associated, in their opinion, with the activity of the gas gland, *i. e.* the nuclei, as well as the cytoplasm, undergo disruptive changes, and that this amitosis (55) leads to the production of new cells which take the place of those broken up! Needless to say, I fail to see any justification for this phœnix-like theory. My own researches have proved that the cells of the gas gland, when worn out like other cells of the body by a long period of activity, are replaced by ordinary mitotic divisions of the small cells at the base of the gland and that amitosis is only found in the later cell-generations. I may also mention in connection with this necrobiotic or decomposition theory of Nusbaum & Reis that, apart from other objections, it has always seemed to me to expect too much from the individual cell of the gas gland. The cell is not only required to commit suicide by undergoing decomposition but whilst undergoing decomposition it is to work energetically† in pumping the results of its own decay into the bladder cavity. Even the cell of the

* This particular objection of Jaeger is invalid since the decomposition of cytoplasm, being irreversible, would not be inhibited by pressure.

† "The formation of the gas bubbles in the cells is accompanied by fragmentation of the nucleus and simultaneous granular disintegration of the cell-plasma. . . . Condensation of the gas [in the cell] must accompany its formation since the lumen of the bladder is filled with gas at a considerable pressure." Translation of Reis (64).

sebaceous gland is not called upon for this degree of self-sacrifice. Finally, the researches of Deineka (29) on the intimate nerve-supply of the cells of the gas gland—it being stated that each gland cell is surrounded by a terminal cluster of nerve-filaments—and the known nervous control of the gas gland by two kinds of fibres running in the vagus and sympathetic nerves respectively (Moreau, 50, 51, 52; Bohr, 20; and others) render it very improbable that such cells undergo the wasteful decomposition assumed by Nusbaum & Reis.

I have now discussed the main features of the rival theories of Jaeger and Nusbaum & Reis. If we eliminate the errors and combine the truths of both with some observations of other authorities, we arrive at the following outline statement of the mode of working of the gas gland. The gas gland, in some unexplained manner, but probably, as Jaeger suggests, by the secretion of a toxin (which Reis & Nusbaum suggest he should have figured!) so influences the blood conveyed to it as to cause the disintegration of a certain proportion of red blood corpuscles. This fact of the disintegration is admitted by Bykowski and Nusbaum & Reis. The disintegration of the erythrocytes is primarily for the purpose of enabling the gas gland cells to “lay hold” of the relatively large quantity of oxygen contained in the blood. Oxygen, in consequence of its easy obtainment from the blood, is the all-important gas required by fishes which undergo considerable and rapid changes of pressure in their bladder consequent on rapid changes in their vertical position, and the gas gland, considered as a special development of the lining epithelium of the bladder, is solely concerned with the rapid production of oxygen. The gas gland being essentially an oxygen gland is therefore most developed in those fishes which possess the greatest proportion of oxygen in their bladder gas; in fishes like the Cyprinidae and some other freshwater families, on the other hand—fishes which exist in inland waters possessing little depth and which cannot, therefore, experience great changes of pressure in their bladder by vertical displacement,—nitrogen and carbon dioxide form the principal constituents of the bladder gas* and gas glands, in the ordinary sense of the word, and retia mirabilia are absent. It was formerly supposed that a large proportion of oxygen in the bladder was associated with the great depth at which the fish existed—the greater the depth the greater the percentage of oxygen,—but apparently this is not altogether the case†. A large proportion of oxygen, as already stated, seems to be, for the most part, associated with the habits of those fish which frequently make considerable excursions in a vertical direction: if the fish sinks, the oxygen gland becomes active and

* *Cyprinus carpio* contains about 94 per cent. of nitrogen in its bladder (Hüfner).

† Cf. *Exocoetus volitans*, the Flying-fish, an essentially surface marine form, possessing, according to Humboldt (41), 94 per cent. of nitrogen in its bladder, with *Coregonus acronius*, a freshwater form living permanently at a depth of over seventy metres and possessing, according to Hüfner, about the same percentage of nitrogen, and many other examples might be quoted.

rapidly produces oxygen in considerable quantity in order to counteract the compression of the bladder due to the increased pressure and so to maintain the equality of the specific gravity of the fish with its medium; if the fish rises, either the absorption-organ known as the "oval" comes into action and returns the surplus oxygen in the swollen bladder to the blood, or, if the fish be a physostome, the pneumatic duct allows the surplus gas to escape to the exterior. Between the two extreme classes of bladder—the oxygen-filled bladder with "red bodies" and the nitrogen-filled bladder devoid of "red bodies"—there exist many transitional kinds containing a relatively small percentage of oxygen and feebly-developed "red bodies"*. As regards the exact mode of abstraction by the gas gland of the gases contained in the blood and their subsequent expulsion into the bladder, papers already published afford little or no information on the subject. In the case of those gases present in the bladder in minute quantities, it is probable that they leave the blood by simple diffusion: *e. g.* Traube-Mengarini (73, 74) and Priefer (60) showed that this was the case when hydrogen was dissolved in the water†. It is evident, therefore, that the problem of gas-abstraction becomes of importance only when the partial pressure of any particular gas in the bladder exceeds that in the blood. That the gas gland cells act as a pump is certain‡; it is also certain that the gases first enter the cells in a dissolved condition and that, just as a scleroblast converts dissolved calcareous salts into a solid spicule, so the gas gland cells cause these dissolved gases to appear in a gaseous form as bubbles in the cytoplasm §. In addition to this, however, the gland cells subject these gas bubbles to a considerable pressure, greater than that existing in the bladder, so that when the gas bubbles are expelled into the bladder lumen and experience a diminution of pressure they burst. As regards the varying composition of the bladder gas, it can only be concluded that the cells of the gas gland, like the cells of the kidney, exercise a selective power. That the oxygen pumped into the bladder is derived from the blood no one doubts, but Nusbaum & Reis, Thilo, and some others find a great difficulty in supposing that nitrogen (and carbon dioxide) is

* *E. g.* *Perca fluviatilis* contains on an average 15 per cent. of oxygen, 83 of nitrogen, and 2 of carbon dioxide (Hüfner); *Lota vulgaris* 65 per cent. oxygen, 30 nitrogen, 5 carbon dioxide (Hüfner), &c., &c. For analyses of the bladder gases in many fish see the works of Biot (19), Configliachi (27), Delaroche (30), Humboldt & Provençal (39, 40), Hüfner (38), Richard (67) among others. The percentage composition of the bladder gas exhibits, as might be expected, considerable variation not only in different individuals of the same species of fish but in the same individual at different times.

† It must be mentioned, however, that Humboldt & Provençal (39) in 1809 performed this experiment of impregnating water with hydrogen and failed to detect a trace of hydrogen in the bladder, but probably their methods of analysis were too crude.

‡ In this connection Hüfner ventures to compare the gas gland cells with the contractile gland cells described by Drasch (32) in the neck-skin of the Frog, both being similarly supplied with nerves.

§ *Cf.* the gas vacuoles in *Arcella* and other Thecamoebida.

similarly derived. It is true that the amount of nitrogen dissolved in the blood is very minute as compared with the amount of oxygen, but, on the other hand, nitrogen does not, like oxygen, require to be produced at a rapid rate; and since the supposition of Nusbaum & Reis, that free nitrogen is generated by the decomposition of cell-substance, is quite inadmissible for the reasons supplied above, the nitrogen dissolved in the blood can be the only source of that gas. It is well known that human blood, when suddenly released from great pressure, develops bubbles of nitrogen owing to the inability of the blood to re-dissolve the gas immediately, and doubtless the cells of the gas gland also so act upon (though certainly not by a diminution of pressure) the blood plasma bathing their substance as to compel the nitrogen to assume a gaseous form. Once abstracted from the blood stream, the nitrogen, in the case of those bladders containing nitrogen at high pressure, is pumped in the same way as, and in many cases with, the oxygen into the bladder lumen*.

I now propose to offer some additional suggestions concerning the physiology of the gas gland which have occurred to me during the course of my work. It is curious that not one of the investigators mentioned in the foregoing pages has attempted to explain in a satisfactory manner the striking conformation of the rete mirabile. A year ago I published in a short note (78) (based, as I have already explained, upon a mistaken conception of the teleost pancreas) a sketch of a new theory concerning the use of the rete which I have again outlined on a preceding page of this paper, but apart from this I have met with no suggestion concerning the physiological significance of the rete mirabile bipolare geminum (or more simply rete mirabile duplex)†. I now propose to restate more fully this theory of mine, but before doing so I will mention the only previous hypothesis of which I am aware. Johannes Müller (53) stated that in his opinion the retia mirabilia associated with the gas glands of teleost fishes (and Müller was one of the first, if not the first, to distinguish the gas glands—"Luftdrüsen"—from the retia mirabilia and to state their proper function as gas-producers) possessed the same utility as the various other kinds of retia mirabilia described by him, viz. to cause the blood stream to flow more slowly for some physiological purpose, and this opinion has been adopted, so far as I know, by all subsequent observers. Presumably the slowness of the blood stream in connection with the gas glands is supposed to be for the purpose of allowing the cells of the gas gland time in which to abstract the gases present in the blood, and that this is one function of the rete mirabile I myself do

* Haldane (36) comes to the same conclusion.

† The following suggestion concerning the use of the duplex rete associated with the gas glands may throw some light upon the physiology of the similar duplex retia mirabilia which are stated to occur in connection with other structures, e. g. the "choroid gland" associated with the eye of many teleosts and the rete in connection with the liver of the Tunny.

not doubt.* But this explanation does not, in my opinion, explain all the facts, since, if mere slowing down of the blood current is the one desideratum, then a rete solely connected with the bladder artery is all-sufficient. Further, it is evident that the explanation takes no account either of the fact that the bladder vein also forms a rete mirabile or of the still more remarkable fact that *the retia on the bladder artery and vein respectively are both formed at exactly the same distance from the gas gland and their constituent arterioles and venules are intimately intermingled as any product of human manufacture purposely so designed could be.* The suggestion which I venture to offer as to the use of this complex arterial and venous rete universally associated with gas glands is the natural conclusion of the following considerations. The fact that some hundreds of the finest capillaries conveying blood to the gas gland are intimately intermingled with and closely apposed to a like number (another significant fact) of similar capillaries conveying blood from the gas gland is suggestive of the exchange between the two sets of capillaries of some substance necessarily of importance to the gas gland with which the rete is connected. This hypothetical substance we must assume both to be diffused from the venous capillaries to the arterial (since diffusion in an opposite direction would not affect the gas gland) and to be derived from the gas gland. The question which next arises is why this hypothetical substance should be poured into the arterial blood before it reaches the gas gland, and the only answer that suggests itself is that it is necessary for this substance to influence the arterial blood in some manner during its passage (made slower by the rete) from the rete to the gas gland, so that by the time the arterial blood reaches its destination, its constitution has become altered. Now I have already stated that the gas gland, in the limited sense of the term, is essentially an oxygen-producing gland and that therefore the oxygen contained in the blood is the one element which the gas gland requires, from which fact we may conclude that the hypothetical substance referred to has something to do with the giving up of oxygen by the blood to the gland. In the foregoing lines I have mentioned that such a hypothetical substance has already been postulated by Jaeger to explain the disintegration of the red blood corpuscles. We may therefore state as extremely probable suppositions that in the rete the venous capillaries contain a relatively large quantity of a toxin poured into the blood by the cells of the gas gland, that this toxin diffuses from the venous capillaries into the arterial, and that whilst the arterial blood is slowly travelling from the

* This function, however, cannot be exercised in the case of the retia mirabilia of the Eel, which are separated from the gas gland by a relatively few large vessels, since the reunion of the arterial capillaries on the side next the gas gland means of course the quickening of the blood stream. Neither can the Eel's rete mirabile possess the supposed function of the "carotid gland" of Amphibia, of "deadening" the heart pulse, since the gill capillary system of the fish must effectively eliminate all trace of this; on the other hand, the rete mirabile must certainly minimize the pressure of the blood supplying the gas gland, a result which, in the present instance, we must regard as a defect.

rete to the gland the toxin effects by a process of hæmolytic the partial or total disintegration of a certain proportion of erythrocytes, the cytoplasm and oxyhæmoglobin pigment of each being scattered in the plasma, the former giving rise to the masses of granular matter so largely present in the blood returned from the bladder and the latter becoming dissolved in the plasma.

Is there any evidence of this process actually taking place? In reply to this question I am able to cite one fact, already stated on page 219 in Part I., which I think constitutes strong evidence in the affirmative. It is evident that if this process takes place then we may expect to find at the proximal pole of the rete, *i. e.* the pole remote from the gas gland, large quantities of granular matter (resulting from the erythrocytolysis above described) present in the venous capillaries but little or none in the arterial, and that towards the distal pole of the rete (the toxin, on the hypothesis, having had time to diffuse from the venous capillaries and influence the arterial blood) granular debris will also appear in the arteries. Careful examination of the rete mirabile associated with active glands has convinced me that this inferred distribution of the granular matter in the rete capillaries is a fact. Figure 32 (Pl. IV.) represents a section taken across the proximal pole of the rete and shows the absence of the erythrocytolytic granules in the arteries; a section across the distal end of the rete, on the other hand, shows that hæmolytic of the arterial blood has commenced, erythrocytolytic granules being present.

Oppel (56) puts a question which will be asked by many other critics of the foregoing theory of "red body" physiology. Why, he asks, is it at all necessary for the erythrocytes to be broken up in order that oxygen may be supplied to the cells of the gas gland? In other words, if, as we know is the case, the blood readily gives up its oxygen to supply the ordinary tissues of the body, where is the necessity for the manufacture of a special toxic substance in order to effect this same liberation of oxygen in the case of the gas gland? I am not aware that Jaeger or any other author has given a definite answer to this question, but a consideration of the facts will soon supply one. In the first place, it is evident that the cells of the gas gland stand in a very different relation to the oxygen of the blood as compared with that of ordinary tissue cells, since whereas the latter are deficient in oxygen and require it solely for purposes of metabolism, the former are already saturated with oxygen and only lay hold of it in order to concentrate it and pump it into the bladder; in other words, the cells of the gas gland require to get a very effectual "grip" on the oxygen which the tissue cells do not*. Secondly, realization of the conditions obtaining in the normal supply of oxygen by the blood to the tissues will enable us to understand the necessity for a toxin. The combination of oxygen with the hæmoglobin

* This necessity for the gas gland cells to be in the closest contact with the blood is possibly accountable for the presence of the intracellular capillaries already described, perhaps also for the intracellular lumina as conveyors of exuded plasma into the cell-substance. The same phenomena are met with in the cells of the liver—an organ which also requires close contact with the blood—the canaliculi representing the intracellular channels.

pigment contained in the red corpuscles is, as is well known, a very loose one, and this loose combination is, according to modern views, only maintained by the maximum partial pressure exerted by the small amount of oxygen dissolved in the blood plasma. Further, it is important to notice in connection with the present subject that in the liberation of oxygen to the tissues of the body, it is the oxygen dissolved in the plasma which is immediately supplied to the tissues, and not the oxygen associated with the hæmoglobin. The oxygen of the oxyhæmoglobin compound only becomes liberated as a consequence of the lowering of the partial pressure of the plasma oxygen caused by tissue absorption and when liberated merely serves to replenish the plasma and is not conveyed directly to the tissues, so that the plasma with its limited solvent action on oxygen must be recognized as the all-important intermediary between the store of oxygen combined with the hæmoglobin and the tissues. Now in the case of the cells of the gas gland, already employed in pumping oxygen into the bladder and certainly possessing all and more than they require for metabolic purposes, it is difficult to imagine that they can absorb the oxygen dissolved in the plasma in the manner employed by ordinary tissue cells. Even if they are so able, the small amount of oxygen so obtainable is quite insufficient for their purposes, since the oxygen, unlike the nitrogen and carbon dioxide, is required to be rapidly produced in large quantities. The only alternative is, then, for the gas gland cells to seize upon the main source of the oxygen, viz. the oxyhæmoglobin, and so obtain in wholesale quantity what the plasma can only supply in retail*. This is effected, as we have seen, by the production of a toxin which, doubtless by a process of hæmolysis, breaks up the red corpuscles into fragments and so liberates the contained oxyhæmoglobin into the plasma, the rete ensuring that this hæmolysis and consequent solution of oxyhæmoglobin in the plasma is effected in time for the dissolved pigment to be available

* This reduction of the blood to the primitive invertebrate condition in which the respiratory pigment is dissolved in the general plasma and not imprisoned in elastic discs (the erythrocytes) as in Vertebrates raises the question as to why, if the former condition enables the tissues to absorb the oxygen more readily, the latter condition has arisen. Apart from a few lamellibranch and other Mollusca and a few Polychætes and *Phoronis* in which hæmatids have been described, all Invertebrates (and, according to Lankester, also Amphioxus and the *Leptocephalus* larva of the Eel, but hæmatids have been stated to occur in the former and possibly exist in small number in the latter) carry the respiratory pigment, when this is present (absent in the tracheate Arthropods, *e. g.*), in the plasma, and it is difficult to understand what advantage accrues from preventing the dissolved oxyhæmoglobin coming into direct contact with the tissues in vertebrate animals. It is of course possible that the indirect distribution of oxygen by way of solution in the plasma conduces to a more even and gradual supply to the tissues, especially in animals like Vertebrates in which most of the tissues are very remote from the limited respiratory area of the body surface; in most Invertebrates, on the other hand, oxidation of the blood takes place over most of the body surface and the tissues are all practically simultaneously reached by the blood which, being contained in sinusoids rather than in capillaries, bathes them on all sides. The more rapid circulation of the blood and larger quantity of hæmoglobin in Vertebrates possibly compensate for the absence of hæmoglobin in the plasma. As is well known, when hæmoglobin is liberated into the plasma in Vertebrate blood, it is at once eliminated by the liver and kidneys. See Addenda (2).

for absorption when the blood reaches the gas gland. Is there any evidence that the cells of the gas gland absorb this oxyhæmoglobin dissolved in the blood plasma? It is not too much to say in reply that every preparation of a gas gland in at all an active condition does provide very substantial evidence as to the actual occurrence of this process. In every such preparation (see figs. 28-31, 50, 68, 73, *e. g.*) it is at once noticeable that the cytoplasm of the gas gland cells in contact with the capillaries—and usually the cells are only separated from the blood by the thin endothelium—is of dense appearance and forms a perivascular zone quite distinct from the rest of the cell-protoplasm, a feature which has been described and figured by all recent observers of gas gland structure—Jaeger, Bykowski, Nusbaum, Reis and the present writer (see Part I.). Nusbaum & Reis hold that this appearance of the cytoplasm next the blood vessels is merely evidence of absorption from the blood of the nutritious matter afforded by the disintegration of the erythrocytes, but for the reasons just given we prefer to believe that the oxygen associated with the hæmoglobin is *the* desideratum of the gland cells and that whatever nutritive value ingested fragments of stroma may possess is quite a minor matter. However, the opinion of Nusbaum & Reis is of value in supporting our conclusion that the cells of the gas gland do actually absorb from the blood material liberated by the breaking-up of the erythrocytes. Examination of good preparations of active glands shows that this darkening of the cytoplasm of the individual gas gland cell situated next the blood-channel really possesses a striped appearance (Pl. IX. fig. 73)—Bykowski & Nusbaum, *e. g.*, describe it as “die charakterische Streifung des Protoplasmas rings um die Blutgefäße”—similar to that seen at the edges of the cells lining portions of the gut, in the Sertoli cells of the testis and in other cases, and this striping found in so many kinds of cells is proof of a process of absorption taking place. We do not suppose that the fragments of corpuscle substance composing the granular matter in the blood stream are absorbed by the gas gland cells, but only the oxyhæmoglobin dissolved in the plasma, and the fact, which we have previously stated, that the veins of the rete mirabile are in active glands full of this granular matter is in accordance with this view. It can thus be proved by what practically amounts to actual demonstration that the cells of the gas gland do absorb the dissolved oxyhæmoglobin directly from the blood (see Addenda (3)), and the natural inference is that this is employed for the supply of oxygen to the bladder.

Judging from analogies provided by other classes of secreting cells, what possibly happens in the metabolism of the gas gland cell in the production of bubbles of oxygen gas from the absorbed oxyhæmoglobin dissolved in the blood plasma is that at that end or pole of the gas gland cell situated next the blood stream the dissolved oxyhæmoglobin forms a loose combination with the cytoplasm, this combination being merely a temporary linkage of the molecules of the two substances (similar, *e. g.*, to the linkage of

side-chains forming "anti-bodies," which Ehrlich assumes to occur in his theory of immunity) and that later at the remote pole of the cell, *i. e.* the region of the cell remote from the blood stream, this loose temporary incorporation of the dissolved oxyhæmoglobin with the cytoplasm breaks down with the liberation of oxygen gas, which, as already seen, arises as bubbles usually in the vicinity of the nucleus. The slight decomposition of cell-substance which we may thus suppose to occur is evidently quite distinct from that decomposition postulated by Nusbaum & Reis. In our supposition the temporary linkage of the oxyhæmoglobin with the cytoplasm is merely to enable the cytoplasm to obtain a "grip" on the oxyhæmoglobin in order to dissociate the oxygen; in the supposition of Nusbaum & Reis it is the cytoplasm itself which decomposes.

A minor point remains to be mentioned. We have already stated that the veins returning the blood from the gas gland contain a relatively large amount of granular matter resulting from the hæmolysis of the red blood corpuscles, and the question remains as to what becomes of this superfluous granular matter. It can only be said in reply that probably a large portion of this disintegration material is eliminated from the blood by the liver, since we know that one function of the liver cells in all Vertebrates is to destroy degenerate corpuscles* and other waste material in the blood; possibly also the spleen assists in this connection, though, judging from its histological appearance, I doubt it. I have also observed in several genera (*Nerophis*, *Gobius*, *Gasterosteus*, &c.) masses of cells lying to the outer sides of or between the kidneys in which a destruction of effete blood-corpuscles seems to be actively proceeding. It is, however, of little concern to us in what manner the blood is clarified in these teleostean fishes; it suffices to say that the relative purity of the arterial blood in the bladder proves that such elimination does take place.

On reviewing the foregoing pages it will be seen that the hypothesis concerning the physiology of the "red bodies" just elaborated has much to be said for it. In its essential features it is the theory of Jaeger and Hüfner, supplemented, however, and in some respects corrected, by the observations of Bykowski, Nusbaum, Reis, and other investigators, and slightly extended by the few suggestions made by the present writer. Its validity is assured by the number of diverse facts which it interprets, for not only does it explain the general fact of the existence of the "red body" as the only mechanism possible under the conditions for the rapid inflation of the bladder, but it also explains the minutest details of this mechanism, such as the perivascular striping of the component cells of the gas gland and the disintegration of the erythrocytes, and such a wide range of interpretation constitutes the criterion of a true theory.

* These erythrocytes in the liver have of course been largely deprived of their oxygen by the gut tissues.

APPENDIX A.

The Sources and Modes of Preparation of Material.

A great part of my material was obtained by me during my occupation of the British Association Table at the Naples Zoological Station during April, 1907; additional specimens were subsequently sent to me from Naples and the remainder I obtained from the Plymouth Marine Biological Station. As regards methods of preparation, I may remark, first of all, that it is important to fix the lining-epithelium of the bladder and its special development, the gas gland, in a distended condition, and this is best effected by puncturing the bladder (*in situ*) posteriorly and immediately filling it with the fixative used, also pouring fixative on the outside of the bladder. By this means all shrinkage of the bladder wall is avoided on removal from the body. Another advisable precaution is to separate the lining-epithelium of the bladder, after it has been well fixed, from the outer layers of the bladder wall, since I found that in many cases the tough muscle and connective tissue composing these latter cause difficulty in obtaining thin sections across the gas gland. The fixatives which I employed chiefly were Zenker's Fluid, Corrosive Acetic, and Mann's Fluid (Distilled Water 100 c.c., Corros. Sub. 2.5 gm., Picric Acid 1 gm., 40% Formol 10–25 c.c.—a fixative which, in conjunction with Borax Carmine and Picro-indigo-carmine, gave me most beautiful results). Some of my specimens were simply fixed in 10% Formol—a fixative which did not yield such good results as the others, though good enough for most purposes. I left objects in Zenker and Mann overnight, but in Corrosive Acetic only for an hour or so. In many cases I fixed duplicate specimens in different fixatives and was thus able to compare results. Objects fixed with Zenker were well washed for several hours with Distilled Water and then, like the remaining objects fixed with other fixatives, graded slowly up to 70% Alcohol (the percentages being in all cases made up with Absolute Alcohol and Distilled Water). Objects fixed with fluids containing Corrosive Sublimate were treated with Iodine at this stage. In most cases I subsequently stained in bulk with Grenacher's Borax Carmine, leaving the objects in for at least twelve hours, and *without differentiation with Acid Alcohol*, dehydrated, cleared, and embedded in Paraffin. The sections (both longitudinal and transverse in many cases), well stained with Borax Carmine, were then stained on the slide with Picro-indigo-carmine*. This stain is made up by adding one part of a saturated solution of Picric Acid in 90% Alcohol (sat. sol. = circa 4.5%) to two parts of a saturated solution of Grubler's Indig-carmin in 70% Alcohol (sat. sol. = circa 1%), and it is well to dilute this stain so obtained with twice its bulk of 70% Alcohol. The sections are placed in this stain for a short time, varying according to the thickness of the sections and depth of staining with Borax Carmine, from a few minutes to over half an hour.

* I am indebted to Mr. A. D. Darbishire for recommending me this stain.

The Picric Acid, of course, differentiates the Borax Carmine, leaving scarlet nuclei, and the Picro-indigo-carmine stains the cytoplasm of the gas gland a dull green, the cytoplasm of red blood corpuscles and secretion products like zymogen granules a brilliant emerald-green, and connective tissue blue. Curiously enough, I did not always obtain these brilliant colour-contrasts with Zenker, especially if the material had been preserved in Alcohol and Glycerine for some time after fixation, but fixation with Mann's Fluid always yielded the best results. Objects which did not stain well with the Borax Carmine I stained with Ehrlich's Hæmatoxylin and Picro-indigo-carmine, and obtained sufficiently good contrasts. The principal object to be attained by using Picro-indigo-carmine is to show up the blood corpuscles and granules. In exceptional cases I employed the Iron-hæmatoxylin method for nuclear details (in *Perca*, e. g.), also Kernschwarz for rendering visible cytoplasmic edges and hence the limits of intercellular and intracellular ducts (in *Gadus*, e. g.). As above mentioned, most of my gas glands were cut both transversely and longitudinally.

APPENDIX B.

On the Artificial Production of Gas Bubbles in Cells of the Gas Gland.

Of the various methods available for compelling fish to produce gas in their bladder I have employed several, but only with one have I succeeded in detecting the gas bubbles in the act of being formed by the cells. For the guidance of others I will first mention my unsuccessful experiments. I first experimented with *Gobius paganellus* at Plymouth, subjecting this fish to increased pressure due to increased depth—one of the easiest methods of inducing gas-production. I took tank specimens from the Biological Laboratory on board the steam launch, and when anchored well out at sea I enclosed three or four specimens in each of five cages. One cage I let down to a depth of 30 feet and kept it there for one hour and a half; a second cage I let down 60 feet for one hour, a third 90 feet for one hour, a fourth 180 feet for half an hour and a fifth 180 feet for two and a half hours. On examining sections of the gas glands of these fish I could detect no decisive differences in the gland cells. I am unable to explain why this experiment was a failure. Possibly even the maximum time allowed ($2\frac{1}{2}$ hours) was too short in which to allow these cells to become active; possibly also the fact that *Gobius paganellus* is a bottom form may have contributed to this negative result. Another experiment which I may mention was to stimulate the two vagus nerves of an Eel (*Anguilla*) with a battery for three hours, the two sympathetics having been cut and the bladder previously emptied and ligatured. At the end of the three hours the bladder certainly contained about one-sixth of its usual volume of gas, but I was unable to detect bubbles in the cytoplasm. I did not try the experiment of injecting fish with Pilocarpine nitrate or any similar drug.

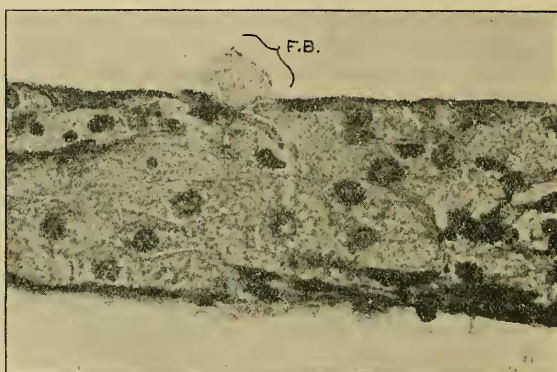
The method by which I achieved success was that of attaching weights to some Perch in a tank in the manner described by Moreau*. The fish, if appropriately weighted, increase the volume of gas in their bladders in order to counteract the sinking action of the weights, but, according to my experience, it is all-important that the weights should not be too heavy, since if the fish are permanently attached to the bottom—if there is no hope of being able to rise—they apparently make no attempt at gas-production. The form of weight I employed was a half-hoop of lead weighing about 5 gm. (approximately one-tenth the weight of the fish, and this was probably too heavy); this I suspended round the lower half of the fish (by thin wires tied dorsally) in the manner of a belt passing dorso-ventrally between the pectoral and pelvic fins, so leaving these perfectly free. After some twelve hours or more the fish can swim about more easily and with a lighter weight it would regain all its usual mobility. If now the weight be removed the fish will rise quite helplessly to the surface of the water, owing to the increased quantity of gas in the bladder. Unless the fish so rises after the experiment it is of little use to examine the gas gland. It is also, of course, necessary to compare the gas gland sections of the fish experimented on with similar sections of a control fish in the same tank. I weighted some six or seven Perch for 17 hours and at the end of that period the bladder of each was appreciably swollen, the fish having to swim vigorously in order to keep below the surface after removal of the weight. I emptied the bladder in the usual manner by a small puncture posteriorly and in several cases refilled it with Zenker's Fluid; in others I used a mixture of one volume of Glacial Acetic with two volumes of Absolute Alcohol, and in others 1% Osmic Acid. Bladders fixed with Zenker I stained with Ehrlich and Picro-indigo-carmin; bladders fixed with the Glacial-Absolute mixture I stained with Iron-hæmatoxylin in order to study the details of nuclear structure; bladders fixed with Osmic I stained with Ranvier's Picro-carmin. In all cases I made surface-view preparations, laying the bladder with its gas gland inside uppermost on the slide, in addition to cutting numerous sections of various thicknesses of the Zenker and Glacial-Absolute material. The Zenker material gave the best results from the gas bubble standpoint, probably owing to the absence of strong diffusion-currents during the process of fixation; the Glacial-Absolute mixture, on the other hand, though perfect in its fixation of most parts of the cell, was doubtless somewhat drastic in its action on delicate structures like extruded gas bubbles.

The sections of the Zenker material showed up the production of the gas bubbles in a very effective manner, the bubbles being clearly seen to arise in the cell-substance (not shown in text-fig. 61, p. 240) and to be extruded as foam-like masses all over the surface of the gas gland lining the bladder (Pl. IX. fig. 70 and text-fig. 61, the latter being a microphotograph). It must be remarked that only occasional cells were thus active, the majority not exhibiting

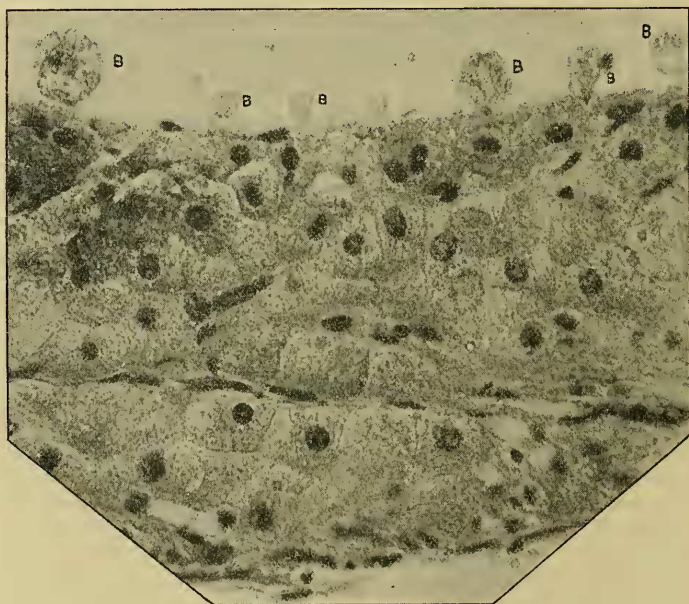
* A more simple method, of course, is to empty the bladder with a trocar.

bubbles. Gas glands must be in a very active condition for the majority of cells to exhibit bubble-structures (see Pl. V. fig. 35 of *Gobius minutus*). These bubbles were quite absent in the sections of non-weighted Perch used for the purpose of controlling the experiment. In my Glacial-Absolute material, structures which are presumably bubbles (very unlike the bubbles seen in most preparations, however) are also to be found in the act of extrusion, but these instead of being unmistakable foam-like masses are bladder-like structures projecting from the cell-periphery (Pl. IX. fig. 71 and text-fig. 62, the latter being a microphotograph) and some are to be found lying free in the bladder-cavity. Possibly the action of the fixative is to cause all the small bubbles of the foam-like mass to run together to form one big bubble. In the surface-view preparations fixed by this method, large gas bubbles are occasionally to be found in the cytoplasm. All these bubble-structures are quite absent in the control material fixed with Glacial-Absolute.

Apart from the existence of gas bubbles in preparations of the activated gland, another distinction from the non-active or dormant gland is the "worked" or "spent" appearance of many of the cells, a distinction which I have tried to indicate in figures 71 and 72 (71 represents cells of the active gland—*cf.* text-fig. 62; 72 represents dormant cells). It was this "spent" appearance, this attenuated and ragged condition of the cytoplasm, which suggested to Nusbaum & Reis that the cell substance actually decomposed into the bladder gases and granular masses, just as the cells of the sebaceous gland disintegrate to produce the secretion of that gland, and, indeed, at first sight of these exhausted cells of the gas gland the suggestion does not seem unreasonable. However, the fact that a cell which possesses but a small amount of protoplasm in the space bounded by its walls is not necessarily in a state of decomposition is shown by the majority of plant-cells, the cytoplasm of which is reduced to a "primordial utricle," by swollen adipose cells, by spicule-cells and by yolk-laden meroblastic eggs, to mention a few out of many possible examples, and since no amount of cytological technique can demonstrate the actual chemical decomposition Nusbaum & Reis assume, this attenuation of the substance of the cells of the gas gland is not of very great significance, especially in view of the reasons for rejecting this suggestion of Nusbaum & Reis already advanced in Part II. It must not be supposed that the cells of my activated glands alone wear this "spent" aspect, all the cells of the dormant gland resembling figure 72; on the contrary, the only difference between the cells of the two glands is one of degree, cells of the dormant gland being, on the whole, less ragged than those of the active. Only a gas gland completely at rest (a condition probably rarely attained in a voracious fish like the Perch) would have all its cells in a resting condition. In most marine fish, however, which I have studied, nearly all the cells of the resting gland have been quite solid in appearance, only the cells of the active gland being attenuated.

Text-fig. 61 ($\times$ circ. 1000).

Microphotograph of transverse section across thin region of gas gland of *Perca* (fixed with Zenker's Fluid), showing foam-like mass of bubbles (F.B.) on surface (cf. Pl. IX. fig. 70).

Text-fig. 62 ($\times$ circ. 1000).

Microphotograph of transverse section across thick region of gas gland of *Perca* (fixed with Absolute and Glacial Acetic), showing extruded gas bubbles (B) on surface (cf. Pl. IX. fig. 71). The "spent" condition of the cells is also shown.

In connection with the two microphotographs of gas bubbles here reproduced as text-figures 61 and 62, I may say that these structures were in my preparations very difficult subjects for photography, partly because of the inappropriate staining employed and partly because bubbles shown in optical section of necessity bear but little resemblance to the real thing (this was especially the case with the foam-like mass indicated in text-fig. 61; *cf.* fig. 70). Nevertheless, these microphotographs, taken for me by Mr. F. J. Pittock, of the Zoological Department, University College, are perhaps of value as affording impartial evidence of the appearance of an active gas gland epithelium, also of the "spent" condition of the cytoplasm. Microphotographs were also taken of intracellular bubbles, but, owing to the thickness and staining of the sections, were not suitable for reproduction.

ADDENDA.

(1) The statement that gas glands are normally quiescent may not be strictly accurate; it would perhaps be more correct to say that only on occasion do gas glands assume *great* activity. I make this remark in consequence of a communication from Lieutenant G. C. C. Damant, R.N., who kindly permits me to repeat it. From observations made under water during diving operations and from the results obtained in catching fish on the hook at different states of the tide, Lieut. Damant concludes that Pout, *e. g.*, remain fairly constantly at one level, *viz.*, just off the bottom, whatever the state of the tide may be. If this be the case, it follows, as Lieut. Damant suggests, that the gas gland must become functional during each rise of the tide in order that a periodic increase of gas in the bladder may counteract the periodic increase of external pressure.

(2) It seems probable, from suggestions kindly made to me by Dr. G. A. Buckmaster and Dr. G. C. Mathison, that the principal factor in the evolution of erythrocytes in Vertebrates has been the necessity for a greatly increased quantity of hæmoglobin in Vertebrate blood as compared with Invertebrate—a necessity corresponding to greater functional activity. It is suggested that the amount of hæmoglobin required to be present in Vertebrate blood would be more than the plasma could possibly hold in solution, and that even if this were possible the viscosity of the plasma produced would seriously interfere with the other functions of the plasma, which, like the respiratory, have, in Vertebrates, increased in intensity. This large quantity of hæmoglobin in Vertebrate blood being requisite and its presence in the plasma prohibited, the evolution of erythrocytes in which the hæmoglobin is imprisoned and combined with a proteid has apparently been the only alternative. As I have before remarked, the lack of direct contact between the tissues and the respiratory pigment in Vertebrates is compensated for both by the rapidity of the circulation and by the amount of the respiratory pigment present, and doubtless also by the increase in amount of the waste

products of active tissues which act as reducing agents and so facilitate the abstraction of the oxygen dissolved in the plasma.

(3) I have adduced the striped darkening of the cytoplasm of the gas gland cells bordering the capillaries as evidence of the absorption of dissolved oxyhæmoglobin from the blood and that this is evidence of absorption is undoubted, but that it is absorption of oxyhæmoglobin is of course only an inference from the hæmolysis of the blood corpuscles, the production of oxygen gas by the gland cells and other considerations. I wish to state here, previous to sending away the final proofs of this paper, that, judging from preliminary experiments with the Israel-Pappenheim stain for hæmoglobin, kindly recommended to me by Dr. G. A. Buckmaster, I have been able to detect the actual presence of hæmoglobin in the cells of the active gas gland and its passage thereto from the blood stream, and Dr. Buckmaster has confirmed these preliminary observations of mine. I intend to repeat these observations on an extended scale on fresh material shortly, and until I have done so I do not wish the above statement to be considered as final.

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EXPLANATION OF PLATES II.-IX.

[All figures of sections drawn with the aid of the camera lucida. In figures 4, 9, 13, 14, 15, 26, 38, 46, 49, 52 and 57, which, like most of the others, have been greatly reduced in size during the process of reproduction, the capillaries of the rete mirabile have not been reduced to nearly the same extent as the other parts of the figures, since, had this been done, they would have been invisible.]

Anguilla vulgaris (Pl. II. figs. 1-8).

- Fig. 1 ($\times 18$). Semi-diagrammatic transverse section through the anterior end of gas bladder (A.B.) and pneumatic duct (P.D.). G.E., glandular epithelium of bladder; D.E., epithelium of duct; A., artery, and V., vein supplying bladder.
- 2 ($\times 18$). Similar section, more posterior. The artery and vein have each divided, each pair of vessels consisting of an artery and a vein.
- 3 ($\times 18$). Still more posteriorly the artery and vein of each pair have subdivided so as to form a mass of intermingled arteries and veins.
- 4 ($\times 18$). Each mass of arteries and veins has, just before the pneumatic duct joins the bladder, subdivided to form a bunch of minute parallel arterial and venous capillaries—the rete mirabile bipolare geminum (Müller) here seen in transverse section.
- 5 ($\times 18$). Where the duct joins the bladder, the minute capillaries of the rete mirabile have reunited to a considerable extent, arteries with arteries and veins with veins, to form large vessels, which then recapillarize in order to supply the gas gland. P.D., pneumatic duct which has now altered the character of its epithelium and become part of the bladder, P.B.
- 6 ($\times 18$). Semi-diagrammatic transverse section through the posterior region of the bladder, P.B.
- 7 ($\times 500$). Transverse section through the lining epithelium of the pneumatic duct. Note the large capillaries and squamous epithelium covering them.
- 8 ($\times 500$). Transverse section through the glandular epithelium of the bladder (gas gland). G.D., one of the gland ducts formed by the folding of the epithelium.

Ophichthys (Sphægebranchus) imberbis (Pl. II. fig. 9).

- Fig. 9 ($\times$ cir. 10). Semi-diagrammatic transverse section through "red body," where the duct (P.D.) joins the bladder (B.). R.M., the single rete mirabile, the long axis of which is situated transversely in the ventral bladder wall. The capillaries of the rete are here seen in longitudinal section.

Syngnathus acus (Pl. II. figs. 10-13; Pl. III. figs. 14-21).

- Fig. 10 ($\times 27$). Semi-diagrammatic transverse section through the anterior attachment of the bladder. A. and V., arteries and veins which subdivide to form the rete mirabile; Ves., vessels not taking part in the formation of the rete; B.G., masses of the diffuse pancreas.
- 11 ($\times 27$). The arteries and veins are here subdividing to form the rete mirabile.
- Figs. 12, 13 ($\times 27$). The formation of the rete mirabile (R.M.).
- Fig. 14 ($\times 27$). The anterior end of the bladder (A.B.) has just appeared. The rete mirabile (R.M.) is fully formed.
- 15 ($\times 27$). The arterial and venous capillaries of the rete mirabile have reunited to form larger vessels which then recapillarize to supply the bladder epithelium (G.E.). B., bladder cavity.
- Figs. 16-18 ($\times 27$). The vessels of the rete mirabile gradually disappear in supplying the bladder epithelium.
- Fig. 19 ($\times 500$). A fold of the "unthickened" bladder epithelium. B.L., gland duct between folds of epithelium; G.S., granular matter present in only very minute quantity; V.D., darkened zone of cytoplasm round capillary wall.
- 20 ($\times$ cir. 1000). Transverse section through rete mirabile. Venous capillaries indistinguishable from arterial. S.G., disintegration-products of the erythrocytes.
- 21 ($\times 1600$). Stages in disintegration of the red blood corpuscles situated in the capillaries of the gas gland. S.G. as in fig. 20.

Gobius niger (Pl. III. fig. 22; Pl. IV. figs. 23-32, 34; Pl. V. fig. 33).

Fig. 22 ($\times 1$). Appearance of the "red body" in the ventral bladder wall.

- 23 ($\times 16$). Semi-diagrammatic transverse section anterior to the "red body."
A. and V., the initial artery and vein which form the rete mirabile;
B.G., pancreas masses; O.L., outermost layer of the bladder wall;
G.E., glandular epithelium lining bladder cavity. It will be
noticed that the vessels and pancreas lie outside the bladder wall.
- 24 ($\times 16$). More posterior section. The initial artery and vein have subdivided
to a small extent. The outermost layer of the bladder wall has
become broken through by the arteries, veins and masses of
pancreas.
- 25 ($\times 16$). The arteries and veins, intermingled with masses of the pancreas,
are rapidly subdividing to form the rete mirabile and the whole
mass is now practically situated inside the bladder wall.
- 26 ($\times 16$). The rete mirabile (R.M.) has now commenced to supply the
glandular epithelium (G.E.) and is situated wholly inside the
bladder wall, *i. e.* internal to the outermost layer (O.L.). B.G.,
portion of the pancreas remaining external to the bladder.
- 27 ($\times 330$). Three folds of the bladder glandular epithelium in transverse
section. C.R.M., minute capillaries of the rete mirabile; BU.,
gas-bubble; IC.C., intracellular capillary.
- 28 ($\times 800$). Gas gland cell with intracellular capillary (IC.C.). Note the
striped darkened zone of cytoplasm next the capillary.
- 29 ($\times 800$). Gas gland cells with inter- and intracellular capillaries (IC.C.).
- 30 ($\times 800$). Gas gland cell with intracellular lumen in transverse section.
Intracellular ducts, when thus seen, are not always easy to
distinguish from bubbles.
- 31 ($\times 800$). Gas gland cell with intercellular lumen (INT.C.L.) in transverse
section.
- 32 ($\times 1000$). Transverse section through anterior end of rete mirabile, showing
the curious endothelium (END.) of the arteries in this region and
the large amount of erythrocyte-disintegration granular matter
contained in the veins. The arteries (A.) are here distinguishable
from the veins (V.) by their smaller size, by their thicker walls, by
possessing the curious endothelium and by being practically free
from granular matter (S.G.).
- 33 ($\times 800$). A duct of the pancreas in longitudinal section. The numerous
nuclei and the syncytial character of the wall are noticeable. A
large amount of granular secretion-matter is present in the duct.
- 34 ($\times 800$). A duct of the pancreas in transverse section.

Gobius minutus (Pl. V. fig. 35).

Fig. 35 ($\times 1000$). Portion of the folded epithelium of the gas gland in a very active
condition. Bubbles are seen being produced (usually near the
nuclei) in the interiors of most of the cells, others are being
extruded from the cells and one is shown lying freely in the
bladder cavity. The large amount of granular matter lying in the
bladder lumen external to the gas gland cells which has resulted
from the bursting of extruded bubbles is noticeable.

Siphonostoma typhle (roudeletii) (Pl. V. fig. 36).

Fig. 36 ($\times 1000$). In addition to the erythrocytes there are shown three of the
curious "white" corpuscles which are so striking in appearance in
this fish, the cytoplasm being very dense. These corpuscles are
numerous.

Peristethus cataphractus (Peristedion cataphractum) (Pl. V. figs. 37-41).

Fig. 37 ($\times 1$). Appearance of "red body" in ventral wall of bladder.

38 ($\times 16$). Semi-diagrammatic transverse section across the anterior end of the
"red body" (see A-B, text-fig. 57, p. 202). S.E., squamous
epithelium lining the greater part of the bladder; G.E., glandular
epithelium; R.M., rete mirabile.

Figs. 39-41 ($\times 1600$). Cells of the gas gland showing formation of bubbles in
cytoplasm.

Trigla hirundo (Pl. V. fig. 43; Pl. VI. figs. 42, 44).

- Fig. 42 ($\times 1$). Lateral "red bodies" (L.R.M.) as seen in ventral wall of bladder (=lateral divisions of the elongated "red body," the median divisions being hidden by the muscle-bands, M.B., in the middle). G.E., the glandular epithelium (too broad in figure).
- 43 ($\times 16$). Semi-diagrammatic transverse section through the middle of the "red body" represented in fig. 42. M.B., two strong muscle-bands on each side of the middle line; R.M., rete mirabile; L.R.M., lateral rete mirabile; G.E., glandular epithelium; S., strand of nerve-fibres supplying muscles.
- 44 ($\times 250$). Glandular epithelium of bladder in transverse section. B.L., gland duct.

Smaris vulgaris (Pl. V. fig. 46; Pl. VI. fig. 45).

- Fig. 45 ($\times 1$). Appearance of "red body" in ventral wall of bladder.
- 46 ($\times 16$). Semi-diagrammatic transverse section across anterior end of "red body." R.M., rete mirabile; G.E., gas gland epithelium; S.E., general squamous epithelium lining bladder; B.L., bladder cavity.

Smaris maurii (Pl. VI. fig. 47).

- Fig. 47 ($\times 250$). Pseudo-massive epithelium of gas gland in transverse section. As explained in the text, this type of epithelium is without doubt a derivative from the folded type and so differs from truly massive types of gas gland epithelia which are not secondarily derived from a folded condition. The perivascular spaces seen in the figure are, in most cases at least, due to contraction.

Ophidium barbatum L. (Pl. VI. figs. 48-50).

- Fig. 48 ($\times 1$). Appearance of "red body" in ventral wall of bladder.
- 49 ($\times 16$). Semi-diagrammatic transverse section across "red body." S.E., squamous epithelium; G.E., gas gland epithelium; R.M., rete mirabile. Outer layers of the bladder wall stripped off.
- 50 ($\times 800$). Large gas gland cells with intracellular ducts (I.C.D.), which possibly represent the passages of bubbles to the exterior. B.L., bladder cavity; INT.C.D., intercellular duct.

Box boops (Pl. VI. fig. 51; Pl. VII. fig. 52).

- Fig. 51 ($\times 1$). Appearance of "red body" in ventral wall of bladder.
- 52 ($\times$ cir. 27). Semi-diagrammatic transverse section through the middle of the "red body" shown in preceding figure. R.M., rete mirabile; S.E., squamous epithelium; G.E., gas gland epithelium; B.L., bladder cavity.

Atherina hepsetus (Pl. VI. fig. 53; Pl. VII. figs. 54, 55).

- Fig. 53 ($\times 1$). Appearance of "red body" in ventral wall of bladder (only the vessels and retia mirabilia are seen).
- 54 ($\times 1000$). The glandular epithelium in transverse section.
- 55 ($\times 1000$). The glandular epithelium of a young *Atherina* (15 mm. long) showing incipient division of the cells in a plane at right angles to the bladder wall. C.T.C., connective tissue cells. Notice the large size of the nuclei as compared with those in the adult gland of another specimen. Though no capillaries are shown in the figure, yet they are plentiful in most parts of the gland.

Coris julis (Pl. VI. fig. 56; Pl. VII. figs. 57, 58).

- Fig. 56 ($\times 1$). Appearance of "red body" in ventral wall of bladder.
- 57 ($\times 16$). Semi-diagrammatic transverse section of "red body." Lettering as before.
- 58 ($\times 500$). The glandular epithelium in transverse section. B.L., bladder cavity; S.E., superficial squamous layer of the glandular mass; BU., gas bubble; INT.C.D., narrow intercellular lumen.

Corvina nigra (Pl. VII. fig. 59).

Fig. 59 ($\times 1$). Appearance of "red body" in ventral wall of bladder.

Sargus rondeletii (Pl. VIII. fig. 60).

Fig. 60 ($\times 1$). Appearance of "red body" in ventral wall of bladder.

Balistes caprisceus (Pl. VIII. fig. 61).

Fig. 61 ($\times 1$). Appearance of "red body" in ventral wall of bladder. The "red body" is, in this case, situated posteriorly in the bladder, not anteriorly.

Zeus faber (Pl. VIII. figs. 62-64; Pl. IX. fig. 65).

Fig. 62 ($\times 1$). Appearance of "red bodies" in ventral wall of bladder. R.M., rete mirabile; G.E., gas gland; M., anterior muscular band.

63 ($\times 375$). Transverse section across base of massive gas gland showing syncytial masses which probably represent the regenerating portion of the gland. Cell-outlines only become visible in the more peripheral regions. The syncytial nuclei divide by ordinary mitosis and are of several sizes, giant nuclei being among them.

64 ($\times 666$). Amitotic division of nuclei in some small giant cells, intermediate-sized cells and small cells of the gas gland. The nucleolus seems to be the first part of the nucleus to divide.

65 ($\times 375$). A binucleated giant cell surrounded by the ordinary small cells. Compare the magnification of this with the last figure.

Gadus morrhua (Pl. VIII. fig. 66).

Fig. 66 ($\times 27$). Semi-diagrammatic transverse section across "red body." R.M., tufts of rete mirabile; G.E., gas gland epithelium; M.C., cap of connective tissue; B.L., bladder cavity.

Cepola rubescens (Pl. VIII. fig. 67; Pl. IX. fig. 68).

Fig. 67 ($\times 1$). Appearance of "red body" in ventral wall of bladder.

68 ($\times 333$). Glandular epithelium in transverse section. The perivascular darkening of the cytoplasm is here very marked. The ducts of the gland are very thin and inconspicuous.

Perca fluviatilis (Pl. IX. figs. 69-72).

Fig. 69 ($\times 1$). Appearance of branched "red body" in ventral wall of bladder, the portions of gas gland forming margins to the small fan-shaped retia mirabilia.

70 ($\times 1000$). A small portion of the gas gland of *Perca* (fixed with Zenker) showing the evolution of bubble-masses from the cytoplasm in certain cells of the epithelium, consequent upon the experiment of weighting the fish (Appendix B, cf. text-fig. 61, p. 240).

71 ($\times 1000$). Cells of the gas gland of *Perca* (fixed with the Glacial-Absolute mixture) exhausted by the activity of the gland (cf. text-fig. 62). One of the bladder-like structures is shown in connection with one of the cells.

72 ($\times 1000$). Unexhausted, *i. e.* inactive cells of the gas gland of *Perca* (fixed by the same method) for comparison with those of fig. 71.

Nerophis aquorinus (Pl. IX. fig. 73).

Fig. 73 ($\times 666$). Five gas gland cells of *Nerophis* in contact with a capillary. This figure, coloured to resemble my preparations as far as possible, shows in the gas gland cell the striped zone of cytoplasm next the capillary wall (indicative of absorption) and the erythrocyte-disintegration granular material present in the blood.